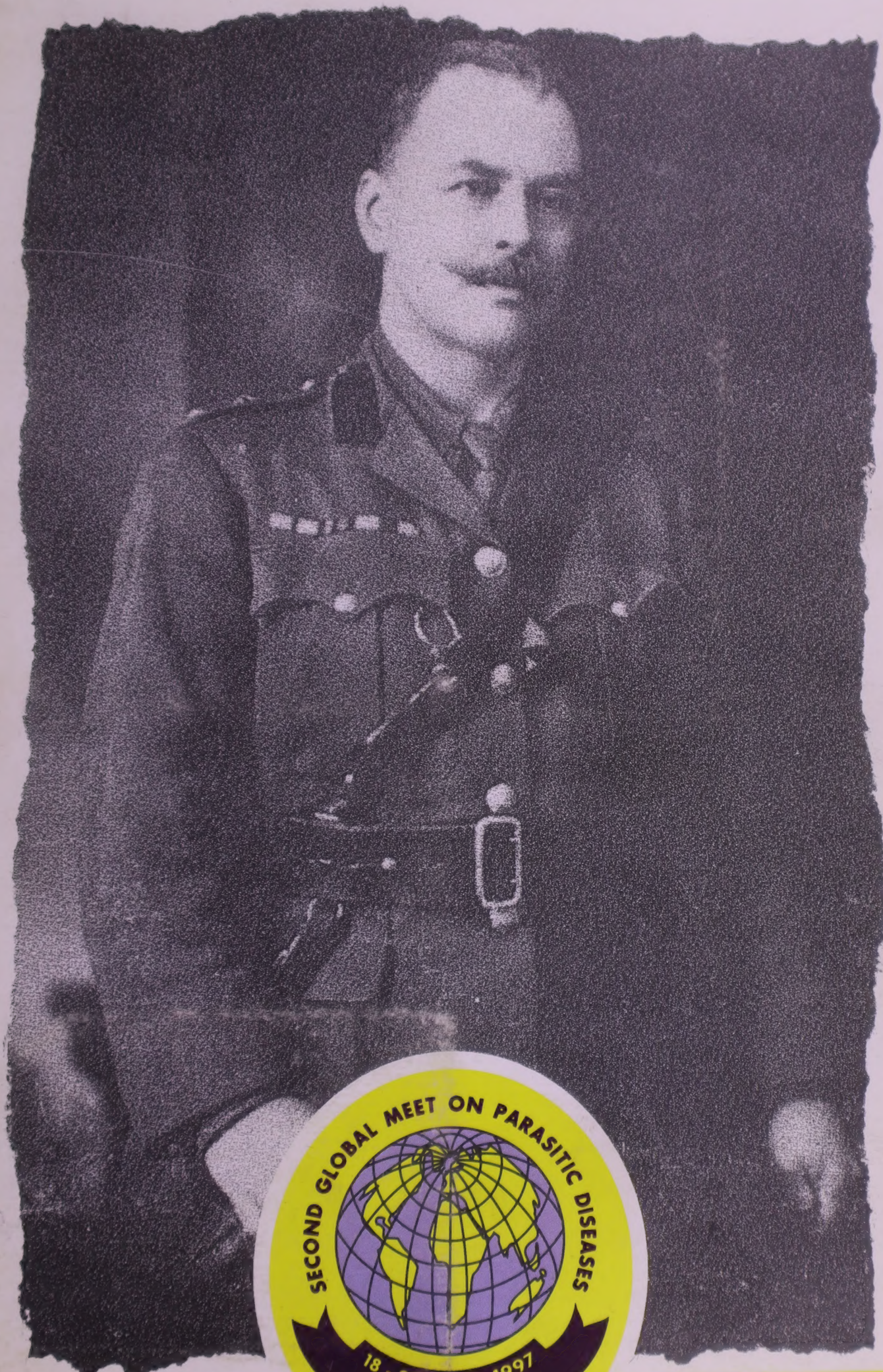

SECOND GLOBAL MEET ON PARASITIC DISEASES

18 - 22 August, 1997
HYDERABAD . INDIA

• ABSTRACT BOOK •

COMMUNITY HEALTH CELL
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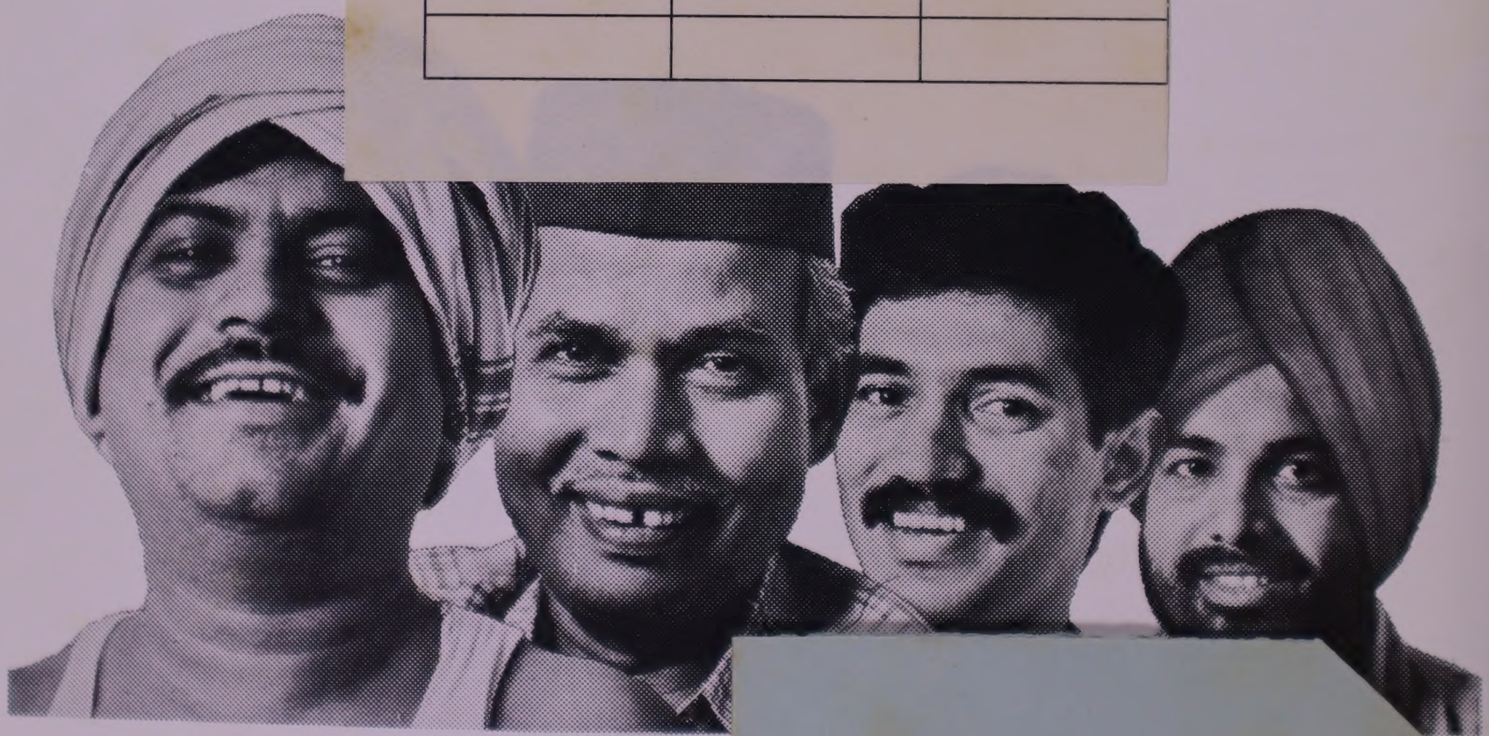

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Second Global Meet on Parasitic Diseases

18 - 22 August, 1997
Hyderabad, INDIA.

Organised by

Indian Society for Parasitology

Celebrating their Silver Jubilee Year

DIS-317 N97
04709

Welcome to
" microcosm of Indian culture "

On behalf of the Indian Society for Parasitology, National and International agencies co - sponsoring the Second Global Meet, and on my own behalf, I have great pleasure in extending a warm welcome to all the delegates and invited guests from India and abroad. A large number of delegates have travelled from long distances to attend this conference, and I am particularly grateful to them for the pains they have taken to be here to attend this conference.

One hundred years ago in August 1897 the aetiology of malaria was discovered by Sir Ronald Ross in Secunderabad. We have for this reason selected the city of Hyderabad - Secunderabad as the venue for the Second Global Meet to commemorate Ross' discovery made in the place he worked. This discovery made Ronald Ross famous throughout the world and he was knighted, awarded the Nobel Prize in Medicine and honoured by a large number of institutions. On the Centenary discovery day of 20th August, a commemorative postage stamp and first day cover would be released. Also on this day Ronald Ross Institute in Secunderabad would be declared open and Ross' statue, a bust and a plaque would be ceremoniously unveiled. A road has also been named " Sir Ronald Ross Road "

Hyderabad has a 400 year colourful history. City offers a large number of tourists attractions - Charminar, Salar Jung Museum, Golconda Fort, Qutab Shahi Tombs, Birla Temple and many more and is also a shopper's paradise and known for minarets and pearls. A large number of restaurant offer some of the best Hyderabadi cuisine. We have made arrangements to offer you some of the choicest dishes served in Royal style. In addition various sightseeing tours and excursions are also available. I hope the arrangements for lodging in various hotels and guest houses are satisfactory and your stay comfortable.

The Conference is being attended by over 750 delegates from at least 25 countries, and almost all states of the Indian Union. Conference scientific programme comprises of a Key Note Address, 5 Plenary Lectures., 30 Symposia, Round Table Conference, Special Sessions, Oral Presentataions and and Poster Sessions. We are looking forward to your participation in making the conference a memorable event.

I welcome you again to this beautiful twin city of Secunderabad - Hyderabad and to this historic conference, and look forward to your active participation in the deliberations of the Second Global Meet.

Dr V P Sharma
Chairman

The Indian Society for Parasitology
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Local Committee - Souvenir
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Ms V Lalitha
Mrs Nirmala Peter

ACKNOWLEDGEMENT

The Indian Society of Parasitology is deeply appreciative of patronage by national and international agencies, financial support and sponsorship by the various institutions to Second Global Meet on Parasitic Diseases.

Co - Sponsorers & Supporters

- Malaria Research Centre, New Delhi
- The National Academy of Sciences, India
- Indian Society for Malaria and other Communicable Diseases
- Indian Council of Medical Research
- Council of Scientific and Industrial Research, New Delhi
- International Centre for Genetic Engineering and Biotechnology, New Delhi
- Defence Research and Development Organization, New Delhi
- National Academy of Medical Sciences, India
- Department of Biotechnology, Government of India
- Department of Science and Technology, Government of India
- Indian Epidemiologists Association
- National Academy of Vector Borne Diseases
- Indian National Science Academy
- The Malaria Foundation Inc., USA
- Medical Research Council, UK
- European Commission
- The Wellcome Trust
- World Health Organization (WHO)
- UNESCO, Paris
- Centers for Disease Control and Prevention
- Burroughs Wellcome Fund
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- Hotel Holiday Inn Krishna, Hyderabad
- Krishna Pearls, Hyderabad

Malaria Foundation Travel Scholarship Program

Made possible with the generous contributions of **Becton Dickinson Worldwide, Abbot Laboratories, Glaxo Wellcome, Bayer Corporation, Pfizer Inc.,** and the **Burroughs Wellcome Fund**

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1 Dr Julie M Moore, USA
2 Dr G Roman Reddy, USA
3 Dr Cheryl Ann Lobo, USA
4 Dr Anjali Yadav, USA
5 Dr Manoj Duraisingh, UK
6 Dr A B Siddique, Sweden
7 Dr H K Alles, Sri Lanka
8 Dr P P S L Pathirana, Sri Lanka

Made possible with the generous contributions of the **Wellcome Trust, UK**

Travel Scholarship recipients :

1 Dr Dilek Turgut - Balik, UK
2 Dr Gehad ElGhazali, Sweden
3 Dr A R Wickramasinghe, Sri Lanka

Index for the Scientific Programme

Plenary Sessions

P - 1	Plenary - keynote address : Scientific Discovery in the Control and Eradication of Malaria	3
P - 2	Plenary - Atomic Force Microscopy and Surface Potential Spectroscopy on *Plasmodium falciparum* Infected Erythrocytes.	4
P - 3	Plenary : The Mosquito Factor in Malaria.	5
P - 4	Plenary : The Intellectual Legacy of Sir Ronald Ross.	6
P - 5	Plenary : Malaria Disease and Mortality at Low Inoculation Rates as Encountered in the Asian Region.	8
P - 6	Plenary : Malaria Control in the World.	9

Symposiums

S - 1	Malaria - The Global Problem.	13
S - 2	Biotechnology in Parasitic Diseases : Leishmaniasis.	15
S - 3	Veterinary Parasitology.	20
S - 4	Vector Biology.	24
S - 5	Natural Immunity to Malaria.	27
S - 6	Biotechnology in Parasitic Diseases.	31
S - 7	Nematodes.	34
S - 8	Molecular Epodemiology of Malaria.	35
S - 9	Challenge & Innovation in Malarial Diagnostic.	39
S - 10	Animal Models for Malaria.	41
S - 11	Biotechnology in Parasitic Diseases.	44
S - 12	Drug Delivery.	47
S - 13	Parasite Biology.	51
S - 14	Malaria Epidemiology, Control and Public Health.	58
S - 15	Drug Resistance in Malaria - case for New Therapeutic.	-
S - 16	Parasite Cultivation.	68
S - 17	Trematodes.	70
S - 18	Future of Malaria Research and Control.	73
S - 19	Vaccine Development.	79
S - 20	Biotechnology in Parasitic Diseases.	83

Symposiums (continued)

S - 21	Adhesins & Protozoans.	87
S - 22	Drug Design & Mechanism.	89
S - 23	Taeniasis & Cysticercosis.	94
S - 24	Clinical Assessment of Vaccines.	97
S - 25	Role of Artemisinin, its derivates and analogues in the chemotherapy of malaria.	100
S - 26	Vector Control - Alternative Approaches	106
S - 27	Round Table Conference on Sterile Insect Technique in Malaria Control.	-
S - 28	Molecular Epodemiology of Malaria.	109
S - 29	Malaria Pathogenesis.	114
S - 30	HIV & Malaria.	119
S - 31	Plant Parasite and Soil Inhabiting Nematodes.	123

Oral Papers

O - 1	Molecular & Cell Biology.	131
O - 2	Parasite Biochemistry & Physiology	141
O - 3	Host Parasite Relationship & Parasite Cultivation	146
O - 4	Drugs & chemotherapy.	150
O - 5	Diagnosis & Clinical Aspects.	159
O - 6	Vector Biology & Control; Public Health.	172
O - 7	Pathogenesis & Epidemiology	182
O - 8	Taxonomy & Life Cycle.	197
O - 9	Vector Biology, Control & Public Health.	202
O - 10	Immunology & Immune Response.	208
O - 11	Ecology & Environment.	221
O - 12	Morphology & Ultrastructure	226
O - 13	Drugs & Chemotherapy	230

Poster Session

PS - 1	Molecular and Cell Biology.	237
PS - 2	Ecology & Environment.	240
PS - 3	Morphology & Ultrastructure.	242
PS - 4	Immunology & Immune Response.	246
PS - 5	Taxonomy & Life Cycle.	263
PS - 6	Socio - economic Research.	269
PS - 7	Drugs & Chemotherapy.	271
PS - 8	Pathogenesis, Epidemiology and Disease Control.	277
PS - 9	Others.	282
PS -10	Biochemistry & Physiology.	284
PS -11	Public Health & Modelling.	288
PS -12	Host Parasite Relationship.	290
PS -13	Drugs & Chemotherapy.	294
PS -14	Parasite Cultivation & Parasite Biology.	306
PS -15	Vector Biology & Control.	311
PS -16	Genetics Biotechnology & IEC.	323
PS -17	Parasite Cultivation & Parasite Biology.	324
PS -18	Zoonoses & Techniques.	330

The Conference activity areas have been designated in the following way

Registration	:	Holiday Inn Krishna (HIK) 17th August - 9am to 9 pm 18th August - 11am to 5 pm
Technical Sessions	:	Holiday Inn Krishna (HIK) Krishna Oberoi (KO)
Exhibition	:	Holiday Inn Krishna (HIK)
Poster Session	:	Holiday Inn Krishna (HIK)
Lunches	:	Holiday Inn Krishna (HIK) Krishna Oberoi (KO)

Conference Halls :

KO

Durbar Hall	-	Sir Ronald Ross Hall
Durbar I	-	Ross A
Durbar II	-	Ross B
Durbar III	-	Ross C
Qutab Shah	-	Ross D
Tana Shah	-	Slide Preview Room

HIK

Kohinoor I	-	Manson A
Kohinoor II	-	Manson B
Pavilion	-	Laveran A
Raga	-	Muller Hall

Help Desk : HIK & KO
17 - 22 August, 1997 from 9am to 5pm

*** We have classified the abstracts according to the scientific channels given in our announcement brochure and selected by authors.*
Presentations have been programmed according to the author's choice & no changes have been made except in few cases, and Author's have been informed accordingly.

Poster Session has been assigned according to the author's choice. Boards available for poster display would be 6ft x 3 ft. Pins would be available in the Muller Hall. Each board in landscape style will allow 2 posters display on one side. Poster size should be 32" x 32" and any larger poster would not be acceptable for display

Monday 18th August, 1997

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-1100	Opening Ceremony	Inauguration	•	•	•	•	•	•	•
1100-1200	Tea								
1200-1300	Session	P - 1	•	•	•	•	•	•	•
1300-1400	Lunch								
1400-1600	Sessions	•	—— S - 1 ——		O - 1	O - 2	S - 2	S - 3	O - 3
1600-1615	Tea								
1615-1830	Sessions	•	S - 4	S - 5	- do -	Press Conf.	S - 6	S - 7	ISP Ex. Com.
1930-2230	Dinner								

* Exhibition and Poster Session at Muller Hall (HIK) from 0900 - 1800 hrs
P - Plenary ; S - Symposia ; O - Oral Paper Presentation

P - 1 Plenary - keynote address : Scientific Discovery in the Control and Eradication of Malaria (Europeon Commission) #
Chair : V Ramalingaswami
Speaker : Louis H Miller

S - 1 Malaria - The Global Problem
Chairs : Brian Greenwood, D Raghunath
Speakers : B Greenwood
V P Sharma
P Alonso
W Kilama
P Singhsivanon

S - 2 Biotechnology in Parasitic Diseases : Leishmaniasis (DBT) #
Chair : Manju Sharma
Speakers : G C Mishra
N Kshirsagar
H K Majumdar
S Arora
C P Thakur
P Das

S - 3 Veterinary Parasitology
Chair : D P Banerjee
Speakers : H S Bali
B P Singh
M S Jagannath
K M L Pathak
J D Ghosh
D P Banerjee

S - 4 Vector Biology (AgrEvo) #
Chairs : Anthony A James, Sarala K Subbarao
Speakers : A della Torre
M Shahabuddin
S K Subbarao
A A James
C F Curtis

S - 5 Natural Immunity to Malaria
Chairs : Altaf Lal, Diane Taylor
Speakers : D Taylor
A V S Hill
S Gupta
A Lal
S Sharma
C Rzepzcyk

S - 6 Biotechnology in Parasitic Diseases : Intestinal Parasites (DBT) #
Chair : V K Vinayak
Speakers : A Bhattacharya
W A Patri
N K Ganguly
V K Vinayak

S - 7 Nematodes
Chair : A B Chowdhury
Speakers : N C Sukul
J M Hawdon
M L Sood
M L Dubey
S Ray

 # sponsored

Monday 18th August, 1997 *(Continued)*

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-1100	Opening Ceremony	Inauguration	•	•	•	•	•	•	•
1100-1200	Tea								
1200-1300	Session	P - 1	•	•	•	•	•	•	•
1300-1400	Lunch								
1400-1600	Sessions	•	S - 1		O -1	O - 2	S - 2	S - 3	O - 3
1600-1615	Tea								
1615-1830	Sessions	•	S - 4	S - 5	O - 1	Press Conf.	S - 6	S - 7	ISP Ex. Com.
1930-2230	Dinner								

* Exhibition and Poster Session at Muller Hall (HIK) from 0900 - 1800 hrs
P - Plenary ; S - Symposia ; O - Oral Paper Presentation

O - 1 Molecular & Cell Biology

Chairs : Shobona Sharma, Y D Sharma
Speakers : P Marta
P Malhotra
S K Arora
Y D Sharma
F A S Kironde
C Qin
R Totiza
P R Bhattacharya
R E Fowler
A Yadava
H Joshi
J Curtis
Neelu Singh
R Madhubala
A Ranjan
D E Lanar
A H Chishti
C A Lobo

O - 2 Parasite Biochemistry & Physiology

Chair : V S Chauhan
Speakers : M Joshi
A Sharma
R R Dhande
P Tazeen
N B Khan
B L Tekwani
S Rathaur
A Das

O - 3 Host Parasite Relationship & Parasite Cultivation

Chair : V K Bhasin
Speakers : B S Das
P Kapoor
H Kaur
N Mehra
P K Bhatnagar

Tuesday 19th August, 1997

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-1000	Sessions	P - 2	•	•	•	•	•	•	
1000-1015	Tea								
1015-1300	Sessions	•	S - 8	S - 9	S - 10	S - 16	S - 15	O - 5	O - 6
1300-1400	Lunch								
1400-1600	Sessions	•	S - 13	S - 14	O - 4	•	S - 11	- do -	- do -
1600-1615	Tea								
1615-1830	Sessions	•	- do -	- do -	- do -	Press Conf.	S - 17	S - 12	- do -
1930-2230	Dinner								

* Exhibition and Poster Session at Muller Hall (HIK) from 0900 - 1800 hrs
P - Plenary ; S - Symposia ; O - Oral Paper Presentation

P - 2 Plenary - Atomic Force Microscopy and Surface Potential Spectroscopy on Plasmodium falciparum Infected Erythrocytes (Wellcome Trust) #

Chair : Louis H Miller
Speaker : M Aikawa

S - 8 Molecular Epidemiology of Malaria

Chair : Karen Day
Speakers : M Bruce
D Walliker
O M Puijalon
C Newbold
C Plowe
A Escalante

S - 9 Challenge & Innovation in Malarial Diagnostics (Becton & Dickinson) #

Chair : H K Webster
Speakers : H K Webster
M T Makler
M Garcia
J B Perrone
S Ray
W K Milhous

S - 10 Animal Models for Malaria

Chair : Allan Saul
Speakers : J A M Langhorne
D Mazier
T V Rajan
S Kumar
K Mendis

S - 11 Biotechnology in Parasitic Diseases : Filariasis (DBT) #

Chair : B C Harinath
Speakers : P K Das
P Kumara Swamy
P Kaliraj
R K Chatterjee
M V R Reddy
S P Pani
S K Kar

S - 12 Drug Delivery

Chair : C M Gupta
Speakers : S K Basu
M K Basu
P K Das
P C Ghosh
C M Gupta
D P Sarkar

S - 13 Parasite Biology

Chairs : A Waters,
G Padmanaban
Speakers : R Sinden
N Kumar
R Menard
A Waters
J W Barnwell
C Chitnis
C Braun - Breton
G Padmanaban
D Roos

S - 14 Malaria Epidemiology, Control and Public Health

Chairs : J P Clark, B Nahlen
Speakers : H Mwenesi
U d'Alessandro
S P Misra
J Cattani
B Sharp
Shiv Lal
M English
N Singh
O Walker
R Steketee
K Patel
C Menendez

S - 15 Drug Resistance in Malaria - case for New Therapeutic Options (SKB) #

(Programme to be announced)

S - 16 Parasite Cultivation

Chair : Kamal K Kamboj
Speakers : C F Golenda
J le Bras
A Krettli
V K Bhasin
R Carter
K K Kamboj

S - 17 Trematodes

Chair : J Mahanta
Speakers : V Southgate
V Tandon
T S K Singh
H Sugiyama
M C Aggarwal
J Mahanta

 # sponsored

Tuesday 19th August, 1997 *(Continued)*

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-1000	Sessions	P - 2	•	•	•	•	•	•	•
1000-1015	Tea								
1015-1300	Sessions	•	S - 8	S - 9	S - 10	S - 16	S - 15	O - 5	O - 6
1300-1400	Lunch								
1400-1600	Sessions	•	S - 13	S - 14	O - 4	•	S - 11	- do -	- do -
1600-1615	Tea								
1615-1830	Sessions	•	- do -	- do -	- do -	Press Conf.	S - 17	S - 12	- do -
1930-2230	Dinner								

* Exhibition and Poster Session at Muller Hall (HIK) from 0900 - 1800 hrs
P - Plenary ; S - Symposia ; O - Oral Paper Presentation

O - 4 Drugs & Chemotherapy
Chairs : V P Kamboj, A Benakis
Speakers : S K Garg
S A Reddy
S R Rameshwaram
Md. Hafeez
M Karkada
J Singh
S Sinhababu
B Das
A V Pandey
B Roy
M Duraisingh
G S Dow
S Nagaichi
N Singh
L Nair
G P Dutta
D Turgut - Balik

O - 5 Diagnosis & Clinical Aspects
Chairs : Neena Valecha, S Ray
Speakers : D Kochar
D C Kaushal
N A Kaushal
B L Kumawat
I Thanvi
S Karan
M N Ali
V Samuel Raj
A Ghosh
A B S Sidhu
A K Tewari
K Singh
A Prakash
Hemaprasanth
S Sharma
S Mohanty
H L Gupta
G N Babu
P N Goswami

O - 6 Vector Biology & Control; Public Health (AgrEvo) #
Chairs : N L Kalra, S K Subbarao, S K Narang
Speakers : M J Chombari
V D Broek
S Poopathi
C J Babu
P Natarajan
N J Shetty
R K Hazra
N Mohapatra
I Kar
N W Beebe
R Tiwari
S Chatterjee
S Rudra
A K Kumar
P Dutta
K R Stark
J Goose
B E Melin
S K Mohanty
S Singh

sponsored

Wednesday 20th August, 1997

PROGRAMME

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-0945	Session	P - 3	•	•	•	•	•	•	•
0945-1010	Tea								
1015-1100	Stamp and First Day Cover Release		Ceremony		•	•	•	•	•
1110-1330	Session	P - 4	•	•	•	•	•	•	•
1330-1415	Lunch								
1415-1610	Session	•	— S - 18 —		•	Press Conf.	•	•	•
1615-1700	Transportation to Ross Laboratory								
1700-1800	Ross Laboratory								
1930-2230	Dinner								

* Exhibition at Muller Hall (HIK) from 0900 - 1800 hrs
P - Plenary ; S - Symposia

P - 3 Plenary : The Mosquito Factor in Malaria
(Malaria Research Centre) #

Chair : D H Molyneux
Speaker : V P Sharma

P - 4 Plenary : The Intellectual Legacy of Sir Ronald Ross
(UNESCO) #

Chair : Peter Lachmann
Speakers : D Bradley
P N Tandon
D Ross
M Dobson
M Gibson

S - 18 Future of Malaria Research and Control

Chairs : Mary Galinski, Marc DeBruycker
Speakers : L Molineaux
A Kitua
Manju Sharma
M Galinski
C Long
P Hagan
B Hanley
J Lamontagne
D Colley
R Feachem

 # Sponsored

Thursday 21st August, 1997

PROGRAMME

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-1000	Sessions	P - 5	•	•	•	•	•	•	•
1000-1015	Tea								
1015-1300	Sessions	•	S - 19	S - 20	O - 7	O - 8	S - 21	S - 25	O - 9
1300-1400	Lunch								
1400-1600	Sessions	•	- do -	S - 22	- do -	S - 27	S - 23	- do -	O - 10
1600-1615	Tea								
1615-1830	Sessions	•	S - 24	- do -	- do -	Press Conf.	S - 26	O - 11	- do -
1930-2230	Dinner								

* Exhibition and Poster Session at Muller Hall (HIK) from 0900 - 1800 hrs

P - Plenary ; S - Symposia ; O - Oral Paper Presentation

P - 5 Plenary : Malaria Disease and Mortality at Low Inoculation Rates as Encountered in the Asian Region (Malaria Foundation) #

Chair : G A T Targett
Speaker : K Mendis

S - 19 Vaccine Development

Chairs : R Anders, V S Chauhan
Speakers : V S Chauhan
A Holder
A Thomas
A Saul
S Hoffman
R Carter
S Jepsen
M Newman

S - 20 Biotechnology in Parasitic Diseases : Parasite Genome (DBT) #

Chair : Alister Craig
Speakers : A Scherf
H del Portillo
M Lanzer
D Lawson
G R Reddy

S - 21 Adhesins & Protozoans

Chair : N K Ganguly, C M Habibullah
Speakers : R C Mahajan
A Lohia
P Das
S Bhattacharya
A Chakravarti
H Kamra

S - 22 Drug Design & Mechanism of Resistance

Chairs : D Krogstad, A Vaidya
Speakers : D Krogstad
A Vaidya
S Looareesuwan
H Vial
P S K Rathod
S K Puri
P J Rosenthal
M Lanzer

S - 23 Taeniasis & Cysticercosis

Chair : R C Mahajan, Nandan Singh
Speakers : C G Cook
P E Dsouza

S - 24 Clinical Assessment of Vaccines

Chairs : B Mons, Lee Hall
Speakers : M M Levine
C Ockenhouse
L Molineaux
C Rogier
P Smith

S - 25 Role of Artemisinin, its derivates and analogues in the chemotherapy of malaria (NASc) ##

Chairs : Nityanand & W Peters
Speakers : S Looareesuwan
A Benakis
S Kumar
K K Bhutani
C W Jefford
C Singh
W K Milhous
N Valecha
O P Asthana

Discussants
Chair : B N Dhawan
N C Nayar
S Misra
A B Balsara
Neeru Singh

S - 26 Vector Control - Alternative Approaches (SICO) #

Chairs : N Becker, G B White
Speakers : A Robinson
C B Beard
S K Narang
N Becker
G B White

S - 27 Round Table Conference on Sterile Insect Technique in Malaria Control (DBT) #

Chair : S Pattanayak
Speakers : N L Kalra
S K Subbarao
C F Curtis
V P Sharma
A A James
V K Vinayak
Shiv Lal
A Robinson
A V Kondrachin
H D Rananaware

sponsored
Vas Dev ## *symposium of*

Thursday 21st August, 1997 *(Continued)*

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-1000	Sessions	P - 5	•	•	•	•	•	•	•
1000-1015	Tea	•	S - 19	S - 20	O - 7	O - 8	S - 21	S - 25	O - 9
1015-1300	Sessions	•	- do -	S - 22	- do -	S - 27	S - 23	- do -	O - 10
1300-1400	Lunch	•	S - 24	- do -	- do -	Press Conf.	S - 26	O - 11	- do -
1400-1600	Sessions								
1600-1615	Tea								
1615-1830	Sessions								
1930-2230	Dinner								

* Exhibition and Poster Session at Muller Hall (HIK) from 0900 - 1800 hrs

P - Plenary ; S - Symposia ; O - Oral Paper Presentation

O - 7 Pathogenesis & Epidemiology

Chairs : B D Das, K Patel, S P Mishra
Speakers : M L Dubey, B Mahajan, R S Yadav, N D Karunaweera, C Sridevi, A Kumar, M B Gravenor, S Premawansa, S Pathirana, H K Alles, K Narain, I Gupta, N Dhingra, N B L Saxena, N C Appavoo, P Bhaskar, J P Verhave, C D Butler, S N Sharma, A K Kapoor, I A Clark, A Scherf

O - 8 Taxonomy & Life Cycle

Chairs : P K Das, R C Dhiman
Speakers : C Kalavati, W Chalobol, P Dorodhy, C Vijayalakshmi, Sunita Sharma, M Khatoon, F Ahmed, A Prasad, M Z Chishti

O - 9 Vector Biology, Control & Public Health

Chairs : T Adak, R S Yadav
Speakers : C Ghish, P K Srivastav, P R Thapar, U D' Alessandro, A Laitonjan, M A Ansari, V D Broel, P N Sehgal, G Narayanaswamy, K Ravikumar

O - 10 Immunology & Immune Response

Chairs : C Chitnis, P Sharma
Speakers : S Chatterjee, H P Gupta, R K Malhotra, K M Bhattacharya, P F Dubois, S Singh, S Dutta, M Sharma, A Roy, S Handunetti, G Ahmad, A Thomas, S Ghosh, D N Rao, G C Varshney, J M Moore, G Elghazali, A B Siddique

O - 11 Ecology & Environment

Chairs : A P Dash, Vas Dev
Speakers : M Woube, A Srivastava, S Prakash, J P Yadav, N S Rao, M Rokkam, U Shameem, R S Salaria, B Colin

Friday 22nd August, 1997

PROGRAMME

Time	Activity	SIR RONALD ROSS HALL	ROSS A	ROSS B	ROSS C	ROSS D	MANSON A	MANSON B	LAVERAN
0900-1000	Sessions	P - 6	•	•	•	•	•	•	•
1000-1015	Tea	•							
1015-1300	Sessions		S - 28	S - 29	S- 30	O - 12	O - 13	S - 31	O - 14
1300-1400	Lunch								
1400-1600	Sessions	Valedictory Function#	•	•	•	•	•	•	•

* Exhibition and Poster Session at Muller Hall (HIK) from 0900 - 1800 hrs
P - Plenary ; S - Symposia; O - Oral Paper Presentation

P - 6 Plenary : Malaria Control in the World (WHO, Geneva) #
Chair : Paul Hagan
Speaker : A V Kondrachin

S - 28 Vector Control (Bayer) #
Chairs : Pushpa J Herath, R Narayanan
Speakers : P R J Herath
J Lines
S K Ghosh
R Narayanan
A R Wickremasinghe
B G J Knols

S - 29 Malaria Pathogenesis
Chairs : M Molyneux, B D Das
Speakers : C Newton
S Rogerson
I A Clark
G Brown
G Grau
O Puijalon
B D Das

S - 30 HIV & Malaria
Chairs : Altaf Lal & S Hira
Speakers : S Hira
R Steketee
B Nahlen
K Campbell
A Lal

S - 31 Plant Parasite and Soil Inhabiting Nematodes
Chair : M S Jairajpuri
Speakers : Q H Baqri
N Mohilal
H K Bajaj
V S Rawat
M Ahmed
W Ahmed

O - 12 Morphology & Ultrastructure
Chairs : G P Dutta, Nutan Nanda
Speakers : P N Goswami
P N Sharma
G P Brennan
P Ramaswamy
L H Bannister
J Sattabongkot
M Dutta

O - 13 Drugs & Chemotherapy
Chairs : O P Asthana, V K Dua
Speakers : S K Mishra
Ola Skold
P Srivastava
N Kshirsagar
K Mohan
A B Balsara

O - 14 Presentation of papers received late

Monday 18th* & Tuesday 19th** August, 1997

Poster Session I : Muller Hall, Hotel Holiday Inn Krishna
Fix Posters on board assigned on date : 17th August, 1997 between 1600 - 1800 hrs
Remove all posters on 20th August, 1997 between 1200 - 1300 hrs

Chairs :	*Forenoon*	*Afternoon*
	B N Nagpal	M A Ansari
	K K Kamboj	S Sinha

PS - 1 Molecular and Cell Biology
Time : 1100 - 1300* & 1400 - 1600**
Presenters:
R S Rane
S Dhiman
K Saravanan
J K Saxena
P S Sizwali
A Rajan

PS - 2 Ecology & Environment
Time : 1600 - 1830* & 1000 - 1300**
Presenters:
K J Linthicum
R C Dhiman

PS - 3 Morphology & Ultrastructure
Time : 1400 - 1600* & 1600 - 1830**
Presenters:
S C Yadav
U Singh
N Nanda
M Johal
Adesh Kumar
C P Singh

PS - 5 Taxonomy & Life Cycle
Time : 1600 - 1830* & 1000 - 1300**
Presenters:
R K Prajapati
N Srivastava
B N Nagpal
S R Patil
R E Hayward
T Singh
Ch. Dhanachand
A K Saxena
T M Bajirao
S V Kharade
R Ahmed
B V Jadav
M G Babre
A H Chisti

PS - 4 Immunology & Immune Response
Time : 1000 - 1300* & 1400 - 1600**
Presenters:
R S Alli
N Chandra
N Tyagi
N Srivastava
H Mustafa
A Kaur
B S Chatterjee
M Jinga
A Puri
P Sharma
A B Siddiqui
G Elghazali
J K Srivastava
S Joshi
P L Prakash Rao
S S Lal
U K Singha
V Ali
S Kulkarni
L Kabilan
N Gupta
A Rodriguez
A Lougovskoi
N Chandra
K Saifullah
N Bhatia
R Chaturvedi

PS - 6 Socio - economic Research
Time : 1400 - 1600* & 1600 - 1830**
Presenters:
M Pai
V S Malviya

PS - 7 Drugs & Chemotherapy
Time : 1000 - 1300* & 1400 - 1600**
Presenters:
D C Purohit
R Sarkar
S Ghosh
P Sarkar
S Anirban
N K Ghosh
A Saha
V P Kumar
L Dama
A K Srivastava
S Chandra
B V Dharmendra
M Singh
S Prabha
A K Nirmala

PS - 8 Pathogenesis, Epidemiology and Disease Control
Time : 1600 - 1830* & 1000 - 1300**
Presenters:
H C Srivastava
Y A Reddy
R L Sharma
L M Lyndem
S Sharma
A M Khan
A Prakash
P K Mohapatra
J S Rao

PS - 9 Others
Time : 1000 - 1300* & 1400 - 1600**
Presenters:
H Prasad
C J Hiware
C S Pant

Poster Session II : Muller Hall, Hotel Holiday Inn Krishna
Fix Posters on board assigned on date : 20th August, 1997 between 1500 - 1700 hrs
Remove all posters on 22nd August, 1997 between 1500 - 1600 hrs

Chairs :	*Forenoon*	*Afternoon*
	A Srivastava	H L Gupta
	P Malhotra	T S Rao

PS - 10 Biochemistry & Physiology
Time : 1100 - 1300* & 1400 - 1600**
Presenters:
D K Kochar
D M N Okenu
A Agarwal
N Goyal
P V Lalitha
A Shukla
S Srivastava

PS - 11 Public Health & Modelling
Time : 1600 - 1830* & 1000 - 1300**
Presenters:
S Haq
S Sridhar
A R Singh

PS - 12 Host Parasite Relationship
Time : 1400 - 1600* & 1600 - 1830**
Presenters:
S K Gakhar
O P Singh
Z Q Bonday
L Mulder
T Adak

PS - 14 Diagnosis & Clinical Aspecs
Time : 1600 - 1830* & 1000 - 1300**
Presenters:
M Singh
S Gupta
P K Murthy
B Sriram
A K Bagchi
S Tiwari
A Dave
M Patnaik
Jyothi M Divya
S Biswas

PS - 13 Drugs & Chemotherapy
Time : 1000 - 1300* & 1400 - 1600**
Presenters:
M Kazim
Naresh Singh
A Zaidi
K Tyagi
A Dube
S Bhatnagar
R Tripathi
F Nigar
U Aseem
R Aggarwal
N J Siddiqi
A Awasthi
K Purbey
P Pal
A K Bhatacharya
P K Kar
D Agarwal
A Caraballo
P Shahi

PS - 17 Parasite Cultivation & Parasite Biology
Time : 1600 - 1830* & 1000 - 1300**
Presenters:
S K Singh
Surman
R Srivastava
S D Srinivas
A Mishra
M Mishra
C R Pillai
K K Kamboj
H Sandhu

PS - 15 Vector Biology & Control
Time : 1400 - 1600* & 1600 - 1830**
Presenters:
R P Shukla
M S Malhotra
S N Sharma
Vas Dev
M H Hodjati
R S Pandian
T S Satyanarayan
R Mohapatra
T Viz
P K Mittal
V K Dua
K Raghavendra
R Kant
S K Sharma
T Adak
K Narang
M K Das
S Wattal
C P Batra
B P Pokhriyal
R Kumari
K R Kumar
R K Mahesh
U S Jyoti

PS - 16 Genetics Biotechnology & IEC
Time : 1000 - 1300* & 1400 - 1600**
Presenters: Venkatachalaiah

PS - 18 Zoonoses & Techniques
Time : 1400 - 1600* & 1600 - 1830**
Presenters:
N L Pal
S Estibeiro

PLENARY

P - 1

SCIENTIFIC DISCOVERY IN THE CONTROL AND ERADICATION OF MALARIA

Louis H Miller
Laboratory of Parasitic Disease, National Institute of Health Bethesda, MD 20892, USA.

Early in the creation of modern India Nehru recognized the importance of science in the country's development. He invested in education and the scientific infrastructure of the nation, accepting the connection between science and control of diseases such as malaria. Science for Nehru and for us today is not limited to the brilliant discoveries of Ross in India and Grassi in Italy around which this meeting is organized, but the contributions of many individual scientists over centuries who developed the ideas which made malaria control possible. Malaria discovery has always been an international effort from the Peruvian Indians and early Chinese herbalists who discovered the precursors of modern drugs to German industry that developed the first synthetic antimalarial drugs before the second world war. It goes from the French scientist Laveran who discovered the protozoan cause for malaria to the recognition of inapparent infections by Nicolle and Sargent and the implications for vaccine development. Today, Australia contributes more per capita to malaria research than any other country and has made a real contribution. More countries need to do their part. With the deteriorating situation of drug resistance and increased mortality, there was the recognition in a recent meeting in Dakar for the necessity of leadership from African scientists in the control of death from malaria in Africa. The meeting in India, focused on one event of 100 years ago, offers a venue to discuss many opportunities for science and to develop the collaborations to respond effectively to the impending disaster.

P - 2

ATOMIC FORCE MICROSCOPY AND SURFACE POTENTIAL SPECTROSCOPY IN *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES

Masamichi Aikawa
Research Institute of Medical Sciences, Tokai University, Isehara, Kanagawa, Japan

The major advantage that the AFM holds over conventional transmission and scanning electron microscopes is that biological specimens do not require fixation and hydration before they can be examined; in other words unfixed specimens in fluid environment can be examined in the AFM. Thus, the long held hope of biologists to be able to study living cells at high resolution has been realized. Studies using the transmission electron microscope (TEM) have shown that cytoadherence of infected erythrocytes to capillary endothelium involves membrane knobs on the red cell surface. These knobs have now been studied with the AFM to determine if there might be some unrecognized factor that plays a role in cytoadherence. TEM has established that the knob on fixed, infected erythrocytes are conical and measures ~ 100 nm at the base and ~ 60nm in height. Section analysis in the AFM revealed that instead of a single protrusion, there are two distinct peaks in each knob; these correspond to binary subunits; the larger is ~60 nm at its base and the smaller is ~40 nm. In other words, instead of ~ 60 nm at its base and the smaller is ~ 40 nm. In other words, instead of consisting of a single protrusion, as appears to be the case in chemically-fixed specimens, each knob comprises a pair of subunits. Clearly, the true architecture of the membrane knobs is effaced by fixation. Surface potential spectroscopy has shown that the knobs are positively charged (+20 mV), although the remainder of the surface of PRBC has a negative charge. The reversal of charge that characterizes the knobs might be due to the aggregation of malarial antigens at the knob surface; if such antigens have a net negative charge of sufficient magnitude, it might prevail locally over the normally positive charge of the erythrocyte membrane. Because the luminal plasma membrane of endothelial cells has a negative charge, the differences in charge between erythrocyte knob and endothelial plasmalemmas might be involved in cytoadherence between the two. Electrical interaction conceivably could sufficiently slow the passage of infected erythrocytes over the endothelium to permit chemical bonds to form between knob antigens and endothelial receptors leading to firm cytoadherence. A similar electrically-based phenomenon may be operative at another stage of the malaria disease process. During erythrocyte invasion by merozoites, the positively-charged apical end of the parasite appears to be attracted to the negatively-charged erythrocyte plasma membrane. This initial electrical event is succeeded by chemical bonding. The importance of electrical phenomenon in biological processes is emphasized by the AFM. A therapy based on disruption of knob electrical charge may be a key to the prevention of cerebral malaria.

P - 3

THE MOSQUITO FACTOR IN MALARIA

V P Sharma
Malaria Research Centre, 22, Sham Nath Marg, Delhi - 110 054, India.

Since the demonstration that mosquitoes transmit malaria by Sir Ronald Ross, man has been making all efforts to control malaria. It is common knowledge that malaria distribution is associated with the occurrence of Anopheles vectors. Ronald Ross himself and later Macdonald used mathematical models to illustrate that vector control has the most profound effect in curtailing transmission, whereas parasite control is supplementary and supportive. The discovery of DDTs residual insecticidal properties provided this opportunity and malaria eradication seemed a realistic goal. Contrary to this, DDT created more problems than it solved. Despite spectacular control of malaria and its control, malaria returned with vengeance, and its control has now become a formidable task. Besides this, use of DDT and other pesticides resulted in the development of insecticide resistance and made vectors largely exophilic and exophagic. As a result, vector control has become difficult, and unattainable in some areas. In addition use of pesticides is hazardous to human health and environment. Eradication strategy is still being followed and many areas have started returning to the original levels of endemicity. The solution lies in local epidemiological response to these emerging challenges. Examples of Indian experience on how malaria has disappeared from some areas and has also taken deep roots in large areas of the country, and how vectors regulate transmission, disseminate drug resistant parasites, colonize man made ecological niches, adapt altered environment and finally emerge victorious would be highlighted.

P - 4.1

THE INTELLECTUAL LEGACY OF RONALD ROSS

David J Bradley
Department of Epidemiology and Population Sciences, London School of Hygiene and Tropical Medicine, Keppel Street, London, WCIE 7HT, UK.

This talk examines the discoveries and ideas of Sir Ronald Ross in malaria and beyond and goes on to assess their impact in the present century and their implications and promise for the next century. The great discovery of the malaria parasite life cycle, for which he is predominantly remembered, was only one of many original contributions from this many-sided genius whose mathematical and literary gifts matched his parasitological skill and perseverance, though sadly not accompanied by tact or commonsense. Within science, his work on epidemiological theory and his concepts of ordered systems within public health are as original as his work on the malaria life cycle where he not only confirmed what others had expected, that malaria parasites could be ingested and developed in mosquitoes, but also more innovatively he demonstrated that the parasites, after development, were also returned to the human host by mosquito bite, which had not been anticipated in the same way. It is perhaps in Ross's coordination of basic science with practical environmental changes for health, and his mixture of conceptual creativity and focus on human needs that Ross has much to teach us for the future.

P - 4.2

P N Tandon
(Abstract not received)

P - 4.3

ANSWERING MALARIA QUESTIONS IN *TOXOPLASMA GONDII* : DRUG RESISTANCE MECHANISMS AND PARASITE CELL BIOLOGY

David S. Roos, Mary Reynolds, Sabine and Robert G.K. Donald
Department of Biology, Univ. Pennsylvania, Philadelphia PA 19104 USA

Among the > 5000 known parasites of the phylum Apicomplexa, *Toxoplasma gondii* is particularly well-suited to laboratory study - providing the opportunity to explore many questions of relevance to malaria in a more experimentally tractable system. Asexual tachyzoites (merozoites) are easily cultivated *in vitro*, permitting automated screens for parasiticidal compounds. Superb ultrastructural resolution facilitates a wide variety of cell biological studies. Sexual crosses are feasible (in cats), permitting classical genetic analysis. Lacking the A/T bias observed in many *Plasmodium* species, recombinant *T. gondii* genes are readily amenable to manipulation and expression in heterologous systems. Molecular genetic tools permit transient and stable transformation, using integrat-

ing or episomal vector, and both high - and low-copy number selectable markers. The molecular genetics of T. gondii is distinguished by two remarkable features : (i) frequencies of stable molecular transformation are sufficiently high for complementation cloning (>1%), and (ii) genomic integration can be accomplished by either homologous or non-homologous recombination (depending on the length of contiguous genomic DNA introduced). Homologous recombination allows targeted gene knock - outs and allelic replacements, while high-frequency nonhomologous recombination permits insertional mutagenesis - the entire parasite genome can be tagged in a single experiment, and tagged loci are readily cloned by plasmid-rescue techniques. Taking advantage of the accessibility of this system, it is possible to address many questions of interest of the malaria.

P - 4.4

M Dobson
(Abstract not received)

P -·4.5

M Gibson
(Abstract not received)

P - 5

THE MACAQUE MODELS OF HUMAN MALARIAS : THEIR USE FOR EVALUATION OF VACCINES

K N Mendis[1], K L R L Perera[1], S M Handunnetti[1] and S Longacre[1]
1. Malaria Research Unit, Faculty of Medicine, University of Colombo, Sri Lanka.
2. United' Immunoparasitologie, Institut Pasteur, Paris, France.

Macaques, old world monkeys indigenous to South East (SE) Asia are natural hosts of several species of simian malaria parasites. They include *Macaca mulatta* (the rhesus) prevalent in much of SE Asia and *Macaca sinica* (the toque monkey) indigenous to Sri Lanka and South India. Of the malaria parasites naturally infecting these macaques, some species are considered to be analogous to human malaria parasites, and in their natural hosts, constitute good models for the study of human malarias. We have used two such natural parasites of *Macaca sinica, Plasmodium cynomolgi* and *P. fragile* as analogues of *P. vivax* and *P. falciparum* malaria respectively in humans based on similarities in morphological and genomic level between the simian and human parasites.

These natural host-parasite pairs have often been overlooked in preference to artificial systems in which the human parasite has been adapted to unnatural hosts (nonhuman primates). Unnatural host-parasite systems tend to produce highly virulent infections, which are very unlike the naturally adapted host parasite systems of humans or monkeys, and may lack relevance to the human infection. We have used two natural parasites of the toque monkey *P. cynomolgi* and *P. fragile* as models of human *P. vivax* and *P. falciparum* respectively for immunological studies, in particular for evaluating the potential of a vaccine candidate of *P. vivax* in humans.

One of the difficulties associated with the development of asexual vaccines against human malaria is identifying candidate antigens for development, and optimizing constructs for clinical testing, because of the lack of appropriate *in vivo* models or of *in vitro* correlates of protection. The Major Merozoite Surface Proteins (MSPI) of *P. vivax* and *P. falciparum* are priority candidates for a human malaria vaccine. The 42 and 19 Kda fragment of C-terminus of MSPI of *P. cynomolgi* expressed in baculovirus was used for immunization of *Macaca sinica* to evaluate their efficacy as models for a human *P. vivax* vaccine. Through consecutive vaccine trials the construct was modified to yield an increasing degree of protection against a blood stage challenge. In the last trial, vaccinated animals developed either no detectable parasitaemia at all (n=2), or a very mild parasitaemias reaching values less than 0.002% which were self cured rapidly (n=4), compared to the control animals (n=6) all of whom developed parasitaemias peaking at 0.6 to 3.8%. These results indicate a high degree of protection in vaccinated animals. Strategic decisions for further development of this candidate vaccine are now being based on experimentation using this model.

P - 6

MALARIA CONTROL IN THE WORLD

A V Kondrachine, P I Trigg
Malaria Unit, Division of Control of Tropical Diseases World Health Organization, Geneva, Switzerland.

Globally the malaria situation is serious and worsening, with an increasing number of epidemics both in epidemic prone areas and in areas previously free of malaria. The problem is increasing due not only to insufficient financial and human resources for control, but also because of land degradation, deforestation and the expansion of agricultural exploitation and mining as populations migrate and countries strive to improve their economics. Furthermore, war and civil unrest contribute dramatically to the malaria burden. Climate changes have also contributed to large scale malaria epidemics in the highlands of Africa and south America. It is estimated that there are 300-500 million clinical cases and 1.5-2.7 million deaths due to malaria each year, 90% of which occur in Africa south of the Sahara.

The worsening problems of resistance of the parasites to the limited number of available drugs to treat or prevent the disease has led to increasing difficulties for decision-marking on their optimal use. This highlights the need to strengthen national capacities for monitoring therapeutic efficacy of antimalarial drugs, for the development of national antimalarial drug policies and research for new antimalarial drugs.

Five year ago in October 1992, the Global Malaria Control Strategy was endorsed by the Ministerial Conference on Malaria Control. It is now being implemented by all malaria endemic countries, except those where civil war and unrest do not allow to do so. The Global Action for Malaria Control, 1995-2000 developed by WHO in collaboration with other UN agencies, gives priority to country support, based on the development of realistic and affordable rational plans of action after the assessment of needs and priorities. Training and operational research are a part of these plans. Emphasis is also placed on better partnership among the national governments, UN organizations and other agencies involved in malaria control.

The plan accords higher priority to malaria endemic countries in Africa south of the Sahara, in view of an urgent need for the establishment of national malaria control programmes and for the implementation of the Strategy at central and district levels. The focus in these countries is on strengthening general health services to provide early diagnosis and prompt treatment at a community level management of severe and complicated malaria and detection and management of epidemics.

In other parts of the world, priority is being given to the reorientation of existing national malaria control programmes in line with the Global Strategy. This includes the strengthening of curative services at all levels of the health care system, the promotion of rational drug use, the development of health information systems, and the selective use of disease prevention, including vector control.

SYMPOSIUM

S-1.1

MEASURING THE GLOBAL BURDEN

Brian Greenwood

Measuring mortality from malaria is difficult because most deaths from malaria occur in communities with few medical resources where most deaths occur at home. In such circumstances, overall mortality rates can be established relatively accurately through active surveillance systems but determining the malaria-specific mortality rate is much more difficult. Attempts have been made to do this using the post-mortem or verbal autopsy technique but with only limited success as deaths from malaria are confused readily with those due to other acute febrile illnesses such as pneumonia.

Hospital inpatient records can provide useful information on the incidence of severe forms of malaria, such as cerebral malaria and, in communities where a high proportion of sick patients attend hospital, hospital statistics may give a reasonably accurate indication of the overall incidence of severe malaria in a community.

Data from clinics and dispensaries provide some information on the incidence of milder forms of malaria but, in many countries, diagnosis of malaria at health clinics is not supported by microscopy. Furthermore, many patients with mild malaria treat themselves at home or attend a private practitioner or a traditional healer. The accuracy of clinic data can sometimes be improved by providing a microscopist but accurate determination of the incidence of mild malaria may require home visits to detect cases who would not present to a clinic (active case detection).

Careful monitoring of the impact of an effective anti-malaria intervention on deaths and febrile illnesses may provide a more accurate measure of the impact of malaria than can be obtained from conventional survey techniques.

S-1.2

THE PROBLEM OF MALARIA IN INDIA

V P Sharma
Malaria Research Centre, 22 Sham Nath Marg, Delhi-110 054.

Historically malaria has been a major public health problem in India. Since independence we have witnessed the near eradication of malaria and its return to unmanageable levels by the primary health care system. The main problems facing malaria control are almost total dependence on insecticides resulting in insecticide resistance, extradomiciliary transmission and ecological succession of vectors. While NMEP was successful in eradication of malaria in 3/4th of the country but transmission continued unabated in the remaining population later engulfing the previously freed areas as well. In the last 3 decades man made malaria has emerged as a major concern and the forma-

tion of various ecotypes. Gradual increase in *P. falciparum,* and build up of drug resistance has further aggravated the problem leading to higher mortality. Return of malaria in various eco-epidemiological zones has attained higher levels of stability. In previously epidemic prone areas malaria epidemics are more frequent with enhanced mortality. Malaria control is attainable but has become transient and complex. Salient features of the current status of malaria and approaches to control would be discussed.

S-1.3

MALARIA EPIDEMIOLOGY IN THE AMERICAS

Pedro L Alonso
Unidad de Epidemiologia Bioestadistica Hospital Clinic (Universidad de Barcelona) Spain.

The malaria endemic areas of the Americas areas have been reducing for the last 50 years, especially so during the malaria eradication programmes of the 50's and 60's. Nonetheless, malaria transmission occurs with varying levels of intensity in large areas of Central and South America.

The epidemiology of malaria is characterized by relatively ineffective vectors, a frequent predominance of *Plasmodium vivax*, low levels of naturally acquired immunity and low to moderate levels of intensity of transmission that lead to large seasonal, year to year and small area variation in disease patterns. Climatic factors such as "EI Nino" have profound effects on the transmission patterns of infection and disease, including the ocurrence of epidemic outbreaks.

A reasonably developed health infrastructure and access to antimalarials, as well as a low transmission of *Plasmodium falciparum* mean that malaria is not a significant cause of death. The age distribution of disease shows that most morbidity takes place in the 20 to 45 year old age group, strongly suggesting that risk of disease is associated with behavioural patterns. Perhaps the best known are that of " garimpeiros " of non-immune miners working in Amazon endemic areas. Malaria is therefore, a major cause of disability in the economically productive segment of the population. In the light of the new international climate favouring malaria research and control, the performance of the latin american emerging economies, and not with standing the logistic difficulties in reaching the populations at risk as well as the growing problem of drug resistance, the perspectives for a continental effort to eradicate malaria from the Americas should be reconsidered.

S - 1.4
W Kilama
(Abstract not received)

S - 1.5
P Singhsivanon
(Abstract not received)

S - 2.1

MACROPHAGE-T CELL INTERACTION IN EXPERIMENTAL VISCERAL LESIHMANIASIS : FAILURE TO EXPRESS COSTIMULATORY MOLECULES ON *LEISHMANIA-INFECTED* MACROPHAGES AND ITS IMPLICATION IN THE SUPPRESSION OF CELL-MEDIATED IMMUNITY

G C Mishra
National Centre for Cell Science, NCCS Complex, Pune University Campus, Ganeshkhind, Pune - 411 007

The most important immunopathological consequence of infection with *Leishmania* seen in murine and human hosts is the suppression of T cell-mediated immune responses to to both mitogens and leishmanial antigens. It has been suggested that this suppression is mediated by macrophages, wither by defective antigen processing and presentation or by the elaboration of suppressive mediators like prostaglandins. Optimum activation of T helper cells requires not only T cell receptor occupancy by the antigen-Ia complex, but also costimulatory signals provided by the antigen-presenting cells. We investigated the status of several costimulatory molecules on infected macrophages from both genetically susceptible BALB/c and resistant C57BL/6 mice. Our results demonstrate that upon parasitization, the macrophages become unable to deliver costimulatory signals to T helper cells, and that this effects is mediated by prostaglandins, as the inhibition of its synthesis by indomethacin recovered the defect. Upon infection with *L. donovani*, B7-1 expression was decreased, while ICAM-1 was marginally increased in BALB/c macrophages and there was no significant change in the expression for B-7 and ICAM-1 in *Leishmania*-infected C57BL/6 macrophages. Expression of VCAM-1 did not change during infection. This selective alteration in the expression of cotimulatory molecules on *L.donovani*-infected BALB/c macrophages was caused by the living parasite, as shown by the fact that killing og the parasites by stibogluconate led to no alteration in the levels of costimulatory molecules. We found that the change in B7-1 expression on the surface of infected macrophages resulted in the inhibition of delayed-type hypersensitivity-mediating functions of T helper cells from BALB/c mice. The results described in this study not only throw light on the possible mechanism of leishmanial pathogenesis, but also open up the possibility of immunotherapy of leishmaniasis by selective manipulation of costimulatory molecules.

S - 2.2

COMPARATIVE TRIAL OF A NEW TREATMENT, CGP 56697 VS. CHLOROQUINE FOR TREATMENT OF ACUTE UNCOMPLICATED *P. FALCIPARUM* MALARIA IN ADULTS IN INDIA

N Kshirsagar, N Gopinathan, N Murthy, M Garg, S Dalvi, A Chogle, J Sorabjee, S Marathe, S nSane, A Bhatt, B Gemperli, I Gathmann and C Royce
KEM Hospital, Kasturba Hospital, Novartis Hindustan, Novartis Basel Switzerland

CGP 56697 is a combination of artemether with a novel compound, benflumetol. One hundred and seventy-nine hospitalised patients (2 centres) with acute uncomplicated *P. falciparum* malaria aged > 16 years were randomised to receive either CGP 56697 (89 patients took 4x4 tablets, each tablet containing 20mg artemether + 120mg benflumetol) or chloroquine (90 patients took 1x4 then 3x2 tablets, each tablet containing 150mg chloroquine sulphate). Both drugs were taken over a 48 hours period. In-patient observation was for 8 days, total follow-up was 29 days. ECG's were performed on days 4, 8 and 29.

CGP 56697 cured 62 of 65 evaluable patients (95.4% 28 day cure rate) whereas chloroquine only cured 15 of 76 evaluable patients (19.7% cure rate, $p<0.001$). There were 3 failures on CGP 56697 : (all RI) as compared to 61 on chloroquine (49 RI, 8RII and 4RII). Parasite reduction at 24 hours was 98.8% (n=89) with CGP 56697; 70.7% (n=89) with chloroquine. Time to fever clearance was 18 hours (n=40) with CGP 56697 and 27 hours (n=54) with chloroquine. Time to gametocyte clearance was 120 hours (n=51) with CGP j56697 and 336 hours (n=63) with chloroquine. All data refer to medians, n = number of evaluable patients.

QTc were not affected by wither treatment. One patient on chloroquine died of cardio-respiratory arrest due to progression to cerebral malaria on Day 6. CGP 56697 was safe and well tolerated and associated with fewer adverse event (AE) reports (i.e. vomiting 1.1% vs 15.6%) than chloroquine.

This trial demonstrated that CGP 56697 provides a rapid, effective and safe treatment of acute uncomplicated *P. falciparum* malaria in adults in India. CGP 56697 is statistically significantly superior in efficacy compared to chloroquine (28 day cure rate 95% vs 19.7%).

S - 2.3

LEISHMANIA DONOVANI : DNA TOPOISOMERASES AS DRUG TARGETS

Hemanta K Majumder
Indian Institute of Chemical Biology, Calcutta - 700 032

Despite tremendous progress made in the understanding of the molecular biology of *Leishmania*, the causative agents for human leishmaniasis, treatment by chemotherapy has seen very little progress in recent years. The toxic pentavalent antimonials still remain the mainstay of treatment for leishmanial infections. The second line of drugs amphotericin B and pentamidine although used clinically are often of limited efficacy and host toxicity. Therefore, improved drug therapy of leishmanial infections is still desirable and rational approaches are needed to identify novel targets which can be expected in these protozoal cells.

Studies from this laboratory have indicated that leishmanial DNA topoisomerases may well provide suitable targets for potential chemotherapy of antimonial drugs. DNA topoisomerases play a pivotal role in modulating the dynamic nature of DNA. They catalyze the breaking and rejoining of either one strand (type I) or both strands (type II) of DNA molecules allowing supercoils to be removed from circular DNAs.

Inhibitors of DNA topoisomerases comprise a variety of structurally diverse compounds that interfere with the DNA nicking-closing activities catalyzed by these enzymes. Clinically active antitumor drugs include inhibitors of topoisomerase I such as Camptothecin and its derivatives and inhibitors of topoisomerase II such as acridines, epipodophyllotoxins and quinolines etc. Some of these compounds also inhibit trypanosomal topoisomerases.

In this study I will present some novel compounds either synthetically derived or isolated from Indian medicinal plants which exert their antileishmanial activities by inhibiting the DNA topoisomerases of *Leishmania*. The mechanism of inhibition of DNA topoisomerases by the compounds will be discussed.

S - 2.4

S Sehgal
(Abstract not received)

S - 2.5

CLINICAL SPECTRA OF KALA-AZAR

C P Thakur
Fraser Road, Patna - 800 001.

Kala-azar which apparently disappeared in 1950's, mainly due to DDT spray done under national malaria Eradication Programme appeared again in 70's with 100,000 cases in 1977 and 250.000 cases in 1991-92. Two small spurts of control measures brought down the spidemic to an endemic level of 15,000-20,000 cases per year.

Kala-azar of Bihar is caused by *Lesihmania donovani* suggestions about *L. tropica* and *L. infantum* as causative agents need confirmation.

Intermittent fever, shivering or rigor, diminution in apetite, loss of weight, splenohepatomegaly, rarely lymphadenopathy, black pigmentation of skin, anemia and leucopenia are the main clinical features. Patients of all age groups are affected but the majority of patients belong to first four decades of life and male outnumbers female, PKDL presents as depigmented macules, redish papules and nodules. Demonstration of L D bodies in splenic or bone marrow aspirates or skin lesions, supplemented with DAT or ELISA and rarely PCR are the main diagnostic method used.

The cure rate with sodium stiboguconate given in the dosage of 20mg/kg bw for 30 days has decreased to 60% and cradiac and other toxicities of the drug have increased. The requirement of pentamidine used as a second line drug to achieve 98%. Cure has increased to 40 injections. A quite sizeable number of patients emerged responsive to both the drugs. For such patients amphotericin B given in the dosage of 1 mg.kg bw daily in 2 hours for 20 days without incremental dose achieved 100%. Cure rate with occasional relapses 3 courses of Amphotericin B has been found more effective in PKDL also than 8-9 courses of sodium stibogluconate.

Oral drugs metronidazole, antiTB drugs, Ketoconazole, cotrimoxazole and even allopurinol have been found ineffective and litefosine is waiting for the trail. Amphotericin B lipid cpmlex has shown excellent results and aminosidine was also found better than sodium stibogluconate.

A combined approach of DDT spray and treatment of cases for 5 year will effectively control the epidemic of kala-azar in Bihar.

S - 2.6

NEOGLYCOPROTEIN CONJUGATED MURAMYL DIPEPTIDE IN THE THERAPY OF VISCERAL LEISHMANIASIS

Pijush K Das
Molecular Cell Biology Division, Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Jadavpur, Calcutta-700 032.

Active targeting of muramyl dipeptide (MDP) to macrophages was studied by conjugation with the neoglycoprotein, mannosyl human serum albumin (Mannose-HSA) using visceral leishmaniasis as the model macrophage disease. Conjugation did not decrease the affinity of the neoglycoprotein for macrophage mannose receptor. Mannose-HSA-MDP was 50 times more efficient than free MDP in inhibiting the growth of *Leishmania donovani* inside peritoneal macrophages. Moreover, in a 60-day murine model visceral leishmaniasis, 95% of spleen parasite burden was reduced by mannose-HSA-MDP at a dose of 0.5 mg/kg/day given for 4 days. Free MDP at a similar dose had very little effect. *In vitro* exposure of MDP caused enhanced generation of O_2 by macrophages whereas generation of NO was not induced. The elevated antileishmanial activity of MDP-treated macrophages in culture was abrogated by O_2 scavengers. In contrast, considerably enhanced amounts of NO and O_2 was generated from macrophages of mannose-HSA-MDP treated animals, and their splenocytes secreted soluble factors providing all the signals required for the induction of NO biosynthesis. Increase in NO production was paralleled by a concomitant increase in antileishmanial activity which was reversed by NO synthesis inhibitors. Splenocyte supernatants treated with anti-IFN-γ or anti-TNF-α antibodies suppressed inducible NO generation by macrophages.

Moreover, intravenous administration of anti-IFN-γ and anti-TNF-α along with mannose-HSA-MDP greatly reduced protection against *L. donovani* infection. Neoglycoprotein-conjugated MDP, therefore, activated mouse macrophages *in vivo* to kill *L. donovani* and this may depend on the physiological generation of NO induced by IFN-γ and TNF-α.

S-3.1

ROLE OF SNAILS IN TRANSMISSION OF PARASITIC DISEASES AND THEIR IMPACT ON ENVIRONMENTAL POLLUTION

H S Bali
Department of Parasitology, P.A.U., Ludhiana

The role of snails in transmission of large number of highly pathogenic trematode disease (Fascioliasis, Paramphistomiasis, Schistosomiasis, Opisthorchisiasis, etc.) of livestock and man, as agricultural pests (*Achatina fulica*) and a cheapest source of proteins, food for coastal populations, poultry feed and calcium supplement are well known. According to Marine Product Export Development Authority (MPEDA), Cochine there is a demand of 20,000 tonnes of edible molluscus in France alone (ICAR, 1985-86).

The developing countries like India are facing with three main problems *viz.*, poverty, population and pollution. Pollution means destruction of purity and contamination is defined as pollution or infections. In general terms excess of substance than normal having biotic effects amounts to pollution. The, biotic or biological factors include all living organisms wherein the activities of organisms influence each others life which consist of interaction between biotic component like producers, herbivores, carnivores and decomposers. Biotic factors are broadly divided as intra and interspecific. Under intraspecific we have to find out the role of mollusca viz., (a) relating of their competition for food, shelter and mate, (b) migration. Under interspecific biotic factors one has to deal with three factors *viz.*, (a) predator and prey relations, (b) associations, (c) reciprocal relationship between snails and water plants for foods.

S-3.2

IMMUNODIAGNOSIS OF HYDATIDOSIS IN DOMESTIC ANIMALS

B P Singh
Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, U.P. - 243 122.

Echinococcosis/hydatidosis is one of the most important zoonotic diseases resulting in the development of large cysts in the vital organs of man and animal in India. Hydatidosis is cosmopolitan in distribution and has the economic importance due to condemnation of organ and carcasses, reduction of milk and meat production, causing infertility in animals and putting restriction on the export of the meat. There is heavy morbidity and mortality when secondary infection is involved. Sometimes, clinical findings are non-specific and mistaken with tumours and tuberculous nodules when the hydatid cysts are localised in the liver and lungs. Under these circumstances, it is imperative to look for a new diagnostic area using different serodiagnostic methods and latest trends in the molecular biological technique and biotechnological tools for the early detection of *Echinococcus* infections and its control.

S-3.3

ACARICIDAL EFFECT OF *STYLOSANTHES SCABRA* AGAINST COMMON TICKS OF DOMESTIC ANIMALS

M S Jagannath
Centre of Advanced Studies, Department of Parasitology, Veterinary College, Hebbal, Bangalore-560 024.

The acaricidal effect of *Stylosanthes scabra* a leguminous plant was investigated. An *in vitro* trial was undertaken with aqueous and methanol extracts of 6th & 7th month old *S.scabra* plants and its efficacy was determined against larval and nymphal stages of *Boophilus microplus, Haemaphysalis intermedia* and *Rhiphicephalus sanguineus* ticks.

Exposure to the aqueous extract at 3.0 and 6.0 per cent levels resulted in cent per cent mortality of *B. microplus* and *R.sanguineus* larvae respectively. Mortality of 56.00 ± 2.00, 54.00 ± 2.00, 41.00 ± 2.00 and 29.50 ± 2.50 occurred at 6 per cent level of *H. intermedia,* nymphs of *R. sanguineus, B. microplus, H. intermedia* respectively. The age of plant seemed to have a relationship with acaricidal effect as mortality of *H. intermedia* and *R. sanguineus* differed and significantly between 6 and 7 month old plants.

Mortality of larval stages varied with the level of concentration of methanol extracts of *Stylosanthes* levels. Highest mortality of 34.50 ± 1.50, 19.50 ± 2.50 and 40.50 ± 0.50 was observed in *B. microplus, H. intermedia* and *R. sanguineus* at 0.6 per cent concentration.

Although mortality of *B. microplus* and *R. sanguineus* nymphs differed significantly no effect was observed on *H. intermedia* nymphs. The age of plant did not result in significant difference in nymphal mortality with methanol extract of *Stylosanthes scabra.*

S-3.4

RECENT ADVANCES IN THE DIAGNOSIS OF *TAENIA SOLIUM* CYSTICERCOSIS

K M L Pathak
Department of Veterinary Parasitology, College of Veterinary & Animal Sciences, Bikaner 334 001.

Cysticercosis, caused by the metacestode of *Taenia solium* though primarily an infection of pigs, is also a serious public health hazard and problem of economic importance in many parts of world including India. In the pig industry, it is responsible for heavy economic losses due to downgrading or total condemnation of affected meat. At present, the routine method for the control is by meat inspection whereby infected carcasses are detected at postmortem when it is too late to save the carcasses from downgrading or

total condemnation and that stage it may be open to abuse and risk of human consumption.

Recent development in molecular diagnostics have immensely aided the diagnosis of this important malady at an early stage. In this paper data are presented on ELISA for detection of anti-cysticercosis antibodies. A new field oriented diagnostic test, the electro-immunotransfer blot technique (EITB) which is based on antigen detection has been developed first time for the early diagnosis of cysticercosis. Four polypeptides (8, 11, 16 and 23kDa) have been identified by SDS-PAGE and EITB and were specifically recognised by pigs with confirmed cysticercosis. DNA hybridization technique for the detection of eggs in human stool sample has been found very sensitive and specific. A dipstick dot-ELISA for detection of *Taenia*-specific coproantigen has been found useful in field studies.

S-3.5

IN VITRO HATCHING AND MAINTENANCE OF *TOXOCARA VITULORUM* SECOND STAGE LARVAE

J D Ghosh
Department of Veterinary Parasitology, College of Veterinary Sciences, CCS Haryana Agricultural University, Hisar - 125 004, Haryana, India.

The tissue phase of *Toxocara vitulorum* cannot be diagnosed by conventional parasitological means. The present study was therefore aimed at developing an appropriate immunodiagnostic test. For this *T. vitulorum* eggs, collected from the faeces of infected buffalo calves and terminal portion of the uterus of adult female worms, were embryonated in 0.5% formalin at 28°C for 20-30 days. Thick outer coats of embryonated eggs were removed by treating with sodium hypochlorite solution at 37°C for 4 hr. After removing the hypochlorite by five washings with sterile normal saline solution (NSS), decorticated eggs were vigorously shaken for 60 sec in a rubber stoppered tube containing sterile sand and NSS. This process yielded more than 98% hatching of second stage larvae (L2). Larvae were cleaned off shell -debris and dead/injured larvae by Baermannisation for 8 hr in antibiotic - antimycotic added sterile Hank's Balanced Salt Solution with nonessential amino acids (HBSS-NEAA) using three layers of coarse cotton cloth. Out of the four media used for maintaining the larvae *in vitro*, RPMI - 1640 could maintain more than 80% viable larvae for 28 days at 37°C and Eagle's Minimum Essential Medium with Hank's Salts (HMEM) for 21 days. In HBSS - NEAA and NSS all larvae were dead by 72 hr and 24 hr respectively. This technique could be used to obtain *I. vitulorum* L2 for antigen preparations, and RPMI - 1640 and HMEM may be used as maintenance media for the L2.

S-3.6

IMMUNODIAGNOSIS OF TISSUE PHASE ASCARIASIS IN BUFFALOES

D P Banerjee
Dept. of Veterinary Parasitology, CCS Haryana Agricultural University, HISAR - 125 004, Haryana

Tissue phase ascariasis cannot be diagnosed by conventional parasitological examinations. Therefore, immunodiagnosis is a method to be followed. Experimental infection of *Toxocara vitulorum* was given in 6-12 months old buffalo calves. The second stage larval somatic (L2 Sm Ag) and excretory-secretory (L2 ES Ag) antigens were used for evaluating double immunodiffusion (DID), counter immunoelectrophoresis (CLEP), indirect haemagglutination (IHA) and indirect enzyme linked immunosorbent assay (ELİSA). The circumlarval precipitin (CLP), bentonite flocculation (BF) and passive cutaneous anaphylaxis (PCA) test were evaluated using only L2 Sm Ag. The DID detected antibodies to L2 Sm Ag and L2 ES Ag in 4 hrs at 28 °C, whereas CLEP gave unexpectedly delayed results. The IHA test detected antibodies at the early stage of infection and titres were much higher with L2 Sm Ag than with L2 ES Ag. The indirect - ELISA gave titres up to 10,240 with L2 Sm Ag and 5120 with L2 ES Ag as early as 7 days post-infection. The BF and CLP were easy to perform and detected the antibody up to 45 days following the last infection. The PCA was employed successfully for detecting tissue toxocarosis in buffaloes for the first time.

S-4.1

GENETIC STRUCTURE OF ANOPHELINE VECTOR POPULATIONS AND THE RELATIONSHIP OF MALARIA TRANSMISSION

Alessandra della Torre
Istituto di Parassitologia, Universit = EO degli studi di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy.

The failures and constraints of antivector measures for malaria control in tropical countries are largely due to the underestimation of the high degree of genetic flexibility and intraspecific variability revealed by anopheline vector species. The unrealistic assumption of a uniform exposure of the vector populations to insecticides is probably the main reason for the unexpected failure of indoor spraying in interrupting malaria transmission. Although now a day full agreement has been reached on the need of vector analysis at intraspecific level, the genetic structure of vector populations has been only marginally explored in few *Anopheles* species, mainly as a by-product of the cytotaxonomic approach based on polytene chromosome observations. These studies allowed to correlate the frequency of some paracentric inversion polymorphisms with variations of physiological and/or ethological characters which may affect relevant epidemiological parameters. Recently available molecular tools have opened new possibilities to analyse the bionomics and the dynamics of vector species. Particular efforts have been made to analyse vector parasite and vector-host relationships, with special reference to the understanding of the molecular basis of susceptibility to *Plasmodium* infections and to the monitoring of the spreading of insecticide resistance. The molecular approach is being also currently used to monitor ecological niche partitioning and natural replacement phenomena among closely related species or intraspecific variants, thus, providing key information for the development of alternative sampling and control methods. Further studies are still needed, particularly in view of the possibility to genetically manipulate vector genome : the practical value of the most sophisticated molecular manipulations will in fact eventually depend on the acquisition of a more detailed knowledge on the ecological genetics of vector populations.

S-4.2

SPECIFIC CELL TYPE IN THE MOSQUITO MIDGUT INVADED BY *PLASMODIUM* OOKINETES

Mohammed Shahabuddin[1] and Paulo Pimenta[2]
1. Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, USA.
2. Universidade Estadual do Notre Fluminense, Centro de Biociencias e Biotechnologia, Rio de Janeiro, Brazil

The crossing of the mosquito gut epithelium is obligatory for malaria parasites to transmit to a vertebrate host. *Plasmodium* ookinetes that fail to cross the epithelium die. Yet,

how the ookinetes interact with the complex repertoire of cells in the gut is not known. It is also not known whether the ookinetes selectively invade a specific cell type in the gut or invade any gut cell without discrimination. To address these questions, we have studied the invasion process *in vitro*. *Plasmodium gallinaceum* (avian malaria parasite) ookinete was allowed to invade isolated sheets of *Aedes aegypti* midgut epithelium. The invaded gut tissues were examined with light microscopy, electron microscopy and laser scanning confocal microscopy. The study showed that the majority of the gut cells which are invaded by *Plasmodium* ookinetes share several common properties: 1) they do not stain with the basophilic dye toluidine blue; 2) they are less osmiophilic - appear electron -translucent with TEM; 3) they are highly vacuolated; 4) their epical surface has very few microvilli; and 5) they over-express vacuolar - ATPase. These cells are present in similar proportions in both the ookinete-infected (17.6%) and the uninfected tissues (19.4%). The results suggest that we have identified a novel cell type in the mosquito midgut, which *Plasmodium* uses to cross the gut tissue. We conclude that *Plasmodium* ookinetes selectively invade these cells, as, similarly, merozoites and sporozoites selectively invade RBC or hepatocytes. We speculate that further study of these cells may identify crucial molecules that are essential for the sporogonic development of *Plasmodium*, and functions of these molecules can be exploited to design novel malaria transmission-blocking strategies.

S-4.3

ANOPHELES FLUVIATILIS RELATED MALARIA IN INDIA

Sarala K Subbarao and Nutan Nanda
Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, INDIA.

An. fluviatilis has a wide distribution in the oriental region and parts of west Asia. It is considered as an important vector in India, Pakistan and Nepal. In India it is widely distributed, although its role in malaria transmission varies from place to place. It is a very efficient vector of malaria in the hills and foothill regions of the north, and the south and also transmits in plains of the south. *An. fluviatilis* has now been identified as a complex of 3 sibling species, species S, T, U. Studies on vector incrimination, host feeding preferences, resting behavior and distribution pattern in different areas in the country have clearly shown distinct differences among sibling species. Malaria situation in the areas where *An. fluviatilis* is prevalent and the differences observed among sibling species will be discussed to enumerate the relationship between malaria incidence and sibling species prevalence.

S-4.4

MOLECULAR ANALYSES OF MOSQUITO-HOST AND MOSQUITO - PARASITE INTERACTIONS

Anthony A James, Brenda Beerntsen, Craig J Coates, Nijole Jasinskiene, Thanh Nguyen, John Peloquin and Ken Stark.

Department of Molecular Biology, and Biochemistry University of California, Irvine
3205 BioSci II Irvine, CA 92697-5930

Key features of vector -borne diseases are the specific associations of an arthropod with both the vertebrate hosts as a source of bloodmeals, and parasites that have evolved to exploit this feeding behavior. We have used molecular genetic techniques to evaluate the genes and products expressed in the salivary glands of mosquitoes that facilitate hematophagy. We have shown that a powerful anticoagulant secreted by the yellow fever mosquito, *Aedes aegypti* targets factor Xa and has primary structure reminiscent of the serine protease inhibitor family of genes. We have been developing transgenic technologies that will allow the introduction into *Ae. aegypti* and *Anopheles gambiae* of exogenous DNAs designed to interfere with parasite development. We are investigating the specific interaction that allow the malaria parasites, *Plasmodium falciparum* and *Plasmodium gallinaceum,* to recognize and invade mosquitoes and host tissues. The latest results of each of these efforts will be presented as well as a brief assessment of the use of molecular tools in vector parasite biology.

S - 4.5

REVIVAL OF RESEARCH ON THE STERILE INSECT TECHNIQUE AGAINST *ANOPHELES*

C F Curits and M H Andreasen
Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, UK.

Research on the sterile insect technique (SIT) against mosquitoes in India was terminated in 1975 after an ill-informed press and political campaign. Since that time Screw Worm Flies and Mediterranean Fruit Flies have been eradicated by SIT from Mexico. *Anopheles stephensi* in South Indian towns is a locally important malaria vector but is reported not to occur in surrounding rural areas. Thus, there appear to be urban "island" populations which are appropriate targets for eradication with SIT after population suppression by environmental management.

A prerequisite for SIT with *Anopheles* is elimination of biting females from batches of males being prepared for release. The existing method for this is translocation of a dominant insecticide resistance gene on to the Y chromosome so that females can be selectively killed by insecticidal treatment early in life. The male linkage of the resistance gene could be stabilised by an appropriately positioned inversion to suppress crossing over. To select such an inversion the methods of Benedict and Chang (1996) are being used to select appropriate markers.

S-5.1

PLASMODIA, PREGNANCY AND THE PLACENTA

Diane Wallace Taylor and Rose F G Leke
Department of Biology, Georgetown University, Washington DC USA & University of Yaounde, Cameroon

Plasmodium falciparum infection may cause serious problems in pregnant women. An overview of the topic will be discussed and data from our recent studies in Yaounde, Cameroon and those obtained by the CDC-group in Kenya will be presented. Questions to be considered may include : Do women who contact malaria during pregnancy have suppressed cellular and humoral responses to malarial antigens ? Does pregnancy influence IgG isotype expression of antimalarial antibodies ? Is the incidence of malaria infection higher in women who have spontaneous abortions and stillbirths compared to those with full-term deliveries ? Is there a correlation between low birth weight infants, the level of TNF in the placenta at the time of delivery, and maternal TNF promoter polymorphism ? Does the cellular composition in the placenta in women i) with and without malaria, ii) who are primigravidae and multigravidae, and iii) Delivering low birth weight or full-term infants differ ? Is the *in vitro* proliferative response of placenta lymphocytes to malarial antigens and mitogens suppressed compared to responses of lymphocytes in the peripheral blood. Does the placental microenvironment contain mainly Th1 or Th2 type cytokines ? Does the presence of malarial parasites influence the profile ? Are cytokine profiles the same in the placenta of low birth weight and full-term infants ? Is there evidence that *P. falciparum* parasites in the placenta are genetically different from those in the peripheral blood ? Certainly, we do not have answers to all of the questions above. It is hoped that the presentation will start a vigorous discussion at the meeting, and that the dialogue will continue after the meeting is over via the internet.

S-5.2

CD8+ CELL RESPONSE TO MALARIA PARASITES

Adrian V S Hill, Magdalena Plebsnaki Joorg Schneider, Michael Aidoo, Carolyn Hannan, Margaret Pinder, Hilton Whittle, Katie Flanagan and Sarah Gilbert.
Nuffild Department of Clinical Medicine, University of Oxford, U.K.
MRC Laboratories, The Gambia.

Direct evidence in murine models of malaria and indirect evidence from field studies of human malaria support a protective role for CD8+ cytotoxic T lymphocyte (CTL) responses against the liver-stage parasite. Extensive field studies in The Gambia have led to the definition of many immunodominant minimal epitopes from pre-erythrocytic antigens for common HLA class I types. Recently new epitopes have been identified in the antigens Exp-1 and LSA-3. Procursor frequencies of such specific CD8+ Tcell are low (1-100 per million peripheral blood lymphocytes) and most epitopes are relatively conserved.

Detailed analysis of CD8+ and CD4+ T cell response to some variable epitopes in the circumsporozoite protein showed remarkable variant specificity and clear evidence of altered peptide ligand antagonism between epitope variants. Mutually antagonistic variant epitopes were found together at high frequency in infected individuals suggesting that antagonism of specific immune responses may be affecting the parasite population structure.

The observed antagonism of natural CD8+ T cell responses supports the development of CTL-inducing vaccines based on epitope strings rather that whole antigens. Recent progress in optimising the immunogenicity of CTL vaccines will be described. A novel prime-boost regime has provided complete CD8+ T cell-mediated protection against *P. berghei* sporozoite challenge in both Balb/c and C57/BL/6 strains of mice.

These recent data provided fresh impetus for the clinical assessment of a new generation of CTL-inducing vaccines against malaria.

S-5.3

FREEZE DRIED DAT ANTIGEN FOR SERODIAGNOSIS OF VISCERAL LEISHMANIASIS - A PRELIMINARY REPORT

Suman Gupta, Suman Tiwari, Ashim Kumar Bagchi, G K Jain and J C Katiyar
Division of Parasitology and Pharmaceutics, Central Drug Research Institute, Lucknow 226 001 (U.P.), India

The laboratory diagnosis of visceral leishmaniasis (VL) presently relies on the demonstration of *Leishmania* amastigotes in bone marrow/spleen aspirates. The procedures are painful, cumbersome and fairly inadequate in early phase of infection.

Of the currently employed tests (IFAT, CIE and DAT), DAT is considered most appropriate and field oriented (the test standardised by us is being marketed as 'LEISHMAN TEST' and used for diagnosis and seroprevalence of kala-azar). The test is simple, economical, field-based technique with higher sensitivity and specificity. The major drawback of the DAT is the limited stability of the liquid antigen at 4°C. In fields where no cooling facilities are available, a stable antigenic formulation would significantly enhance its practical utility. With this aim we have developed a freeze dried antigen (FD antigen) for the detection of VL using specific freeze drying solution. Initial studies carried out with different categories of antileishmanial sera depicted that FD antigen is comparable in reactivity to liquid DAT antigen and has the major advantage of (i) long shelf life (ii) easy packaging and transportation (ii) reproducibility in results over a long period of time due to production of a large batch of antigen (iv) storage of ambient temperature.

S-5.4

MALARIA INFECTION AS A CO-FACTOR IN HIV INFECTION AND REPLICATION

Altaf A Lal
Division of Parasitic Diseases, National Centre for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Ga-30341

Over half of the HIV-1 infections in the world occur in sub-Saharan Africa. More than 90% of the world's 300-500 annual malaria cases also occur in this region. In addition, circumstantial evidence indicates that progression from HIV infection to AIDS in malaria-endemic areas may be substantially faster than in nonmalarious regions. Because malaria infection activates mononuclear cells and stimulates the production of proinflammatory cytokines, a possibility exists that malaria infection may promote HIV-1 infection and replication. In order to understand the interaction between malaria and HIV at molecular level, we have investigated the effect of malarial antigens on *in vitro* infection of unstimulated peripheral blood mononuclear cells (PBMC) with HIV-1. Stimulations with both soluble malarial antigens and purified malarial pigment significantly enhanced HIV-1 replication in CD8-depleted PBMC from both CCR-5+/+ as well as CCR -5-/- (homozygote for 32 bp deletion) donors, when compared with untreated PBMC. The stimulatory effect was malarial antigenspecific, since control erythrocyte antigens or proteinase-treated pigment did not result in enhancement of HIV-1 replication. Both malarial preparations mitogenically activated PBMCs from healthy donors as judged the expression of cell activation markers, proliferative responses, and cytokines production. Furthermore, neutralizing mAb to TNF-α inhibited malarial antigen or pigment-induced HIV-1 replication, whereas neutralizing mAb to IL-6 had no significant effect. Malarial preparations also increase HIV-1 replication by activating LTR-directed viral transcription. The results of this in vitro study demonstrate a profound modulation of HIV replication by malarial antigens, and suggest the malaria infection can modulate HIV-1, pathogenesis both by promoting the infection through the activation of lymphocytes, and by increasing viral replication through the production of cytokines. We caution, however, that interpreting this data to link malaria with rapid emergence of AIDS be held in abeyance until field-based immunoepidemiologic investigations are conducted to address this issue.

S-5.5

PREVALENCE OF *GONODES DISSIMILIS* DENNY (PHTHIRAPTERA : ISCHNOCERA) INFESTING POULTRY BIRDS *GALLUS DOMESTICUS*

Sangeeta Sharma[1], S K Singh[1], Surman[1], A Kumar[2] and A K Saxena[1].
1. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Kotdwar (Pauri Garhwal), India.
2. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Rishikesh, (Dehradun), India.

Prevalence rate of poultry fluff louse, *Gonioddes dissimilis* on the poultry birds belonging to 18 localities of Dehradun has been found to be quite low (4.0%, n = 1200). Intensity of infestation has been noted by coding system. Most of the infested birds could be placed in very light infestation category (62.5%), followed by light and moderate (16.7% and 12.5% respectively) infestation category. Heavy/very heavy infestation has been found on only 6.25% and 2.1% birds. Impact of host sex, feather colour, feather condition, health and hygienic condition on prevalence rate has also been taken into consideration.

S-5.6

IgG SUBCLASS ANTIBODIES TO MSA2

Christne M Rzepozyk, George Latham, Owen Tak Ying Tsang, Alison Stewart, Elizabeth Hoban and Antonio Ferrante
The Queensland Institute of Medical Research and Australian Centre for International and Tropical Health and Nutrition, Herston Brisbane Australia
Department of Immunopathology, the Women's and Children's Hospital, Adelaide, Australia

Human IgG comprises four subclasses (IgG1 to IgG4) associated with specific functional attribute. We investigated the naturally acquired IgG subclass response to the merozoite surface antigen 2 (MSA 2 or MSP2) of *Plasmodium falciparum* in a group of adult Solomon Islanders and in children 6 months to 5 years of age. MSA2 is a vaccine candidate antigen in which there is considerable current interest because it is undergoing clinical trials. We observed that a high proportion of adult Solomon Islanders had antibodies to MSA2, with high titres being obtained in many individuals particularly to the FCQ-27 allelic form of the antigen. In children high titres to this antigen were already observed in 5 year olds. Strikingly though, the anti-MSA2 response in both groups was primarily confined to the IgG3 subclass suggesting an absence of downstream switching of the response to IgG1. We have proposed that while these IgG antibodies may be very effective in anti-parasite immunity because of there complement fixing, phagocytic and ADCC capacity, the response may rapidly wane in the absence of re-stimulation. This is because IgG3 antibody responses are normally short-lived. The presence of high titres of anti-MSA2 antibodies in children needs further evaluation particularly with regard to the relationship of these antibodies to the acquisition of protection.

S-6.1

PREDICTION OF ANTIMALARIAL POTENCY USING CALCULATED MOLECULAR ELECTRONIC PROPERTIES

Apurba K Bhattacharjee, Tweeny R Kau and Jean M Karle
Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC, USA

Quantum chemical computations on a series of mefloquine analogs reveal correlations between specific molecular electronic properties and *in vitro* antimalarial activity providing a set of discriminators of antimalarial efficacy. The 3-dimensional structures of 21 mefloquine analogs were optimized using the semi-empirical AMI method. Electronic properties such as dipole moment, molecular electrostatic potentials (MEPs) and highest occupied molecular orbitals (HOMOs) were calculated. The most efficacious compounds have a dipole moment pointing towards the quinoline nitrogen atom. Profiles of negative MEP beyond the Van der Waals surface reveal that a large laterally extended region in the frontal portion of the quinoline ring and an absence of the potential over the aromatic ring for the CF_3-containing compounds are linked to potent activity. The MEP profile at the Van der Waals surface shows that the site for the most positive potential lies by the hydroxyl proton whereas the site for the most negative potential is either by the piperidyl or the quinoline nitrogen atoms. A negative potential in the range - 62 to -55 kcal/mol by the aliphatic nitrogen atom and a positive potential greater than 35 kcal/mol by the hydroxyl proton appear to be two important features for potent activity. Proton affinity of the aliphatic nitrogen atom indicates that a value > 218 kcal/mol does not favour potent activity. HOMO energies are more negative for the more potent compounds containing electron-withdrawing substituents like CF_3 on the quinoline ring. Overall, electron-withdrawing substituents at specific positions and intermolecular hydrogen bond formation appear to be two crucial features responsible for *in vitro* activity whereas increased lipophilicity and nucleophilic susceptibility of the aromatic ring together with intermolecular hydrogen bond formation contribute to *in vivo* activity as reported by us earlier. These observations illustrate the fundamental molecular forces that affect the function and utility of the compounds and therefore, should aid in the designing of new antimalarial compounds.

S-6.2

ADHERENCE AND CYTOLYSIS BY *ENTAMOEBA HISTOLYTICA*

William A Petri
University of Virginia, Charlottesville VA 22908, USA

Entamoeba histolytica trophozoites have a surface lectin that recognizes GaINAc/Gal glycoconjugates on colonic mucins and host cells. GaINAc or Gal block adherence of trophozoites, and thereby prevent amoebic contact-dependent killing of host cells. The GaINAc/Gal lectin is a 260 kDa heterodimer of transmembrane heavy and lipid-an-

chored light subunits encoded by a multi-gene family. The heavy subunit contains areas with limited sequence identity to plant lectins and to the human complement regulatory protein CD59. Monoclonal antibodies against the GaINAc/Gal lectins have been used to define functional domains involved in carbohydrate binding, cytotoxicity, and inhibition of human complement C5b-9 membrane attack complex assembly. A major goal of our laboratory is to genetically validate to role of the lectin in virulence. The recent development of DNA transfection systems by our lab and others has made this possible. Lectin structure and function are being probed in trophozoites by expression of antisense RNA and dominant negative mutants. Studies are planned to address the importance of the cytoplasmic and extracellular domains in adherence, cytotoxicity, and serum resistance.

Clinical applications of research on the lectin include the development of a new diagnostic test. Current diagnosis of amoebiasis (by microscopic identification of cysts and trophozoites in stool samples) is insensitive and unable to distinguish pathogenic *E. histolytica* from nonpathogenic *E. dispar*. We have used monoclonal antibodies specific for the GaIN Ac/Gal lectin to detect *E. histolytica* in stool samples from children with colitis. Compared to the gold standard of culture, the antigen detection test is more sensitive than microscopy, and able to differentiate *E. dispar* from *E. histolytica* with 95% sensitivity and 93% specificity. Antigen detection tests for *E. histolytica* should facilitate diagnosis, as well as enable studies of the epidemiology and natural history amoebiasis.

S-6.3

PROBLEM OF SYMPTOMATIC AND ASYMPTOMATIC AMOEBIASIS

N K Ganguly, Ajaib Singh, Anuradha Chakarvarti and R C Mahajan
Department of Experimental Medicine & Biotechnology and Parasitology Postgraduate Institute of Medical Education & Research, Chandigarh, India.

Diamond *et al.* have recently proposed a hypothesis that the genus *Entamoeba* exists in two forms. One of which is *E. histolytica* which causes diarrhoea/dysentery and another as *E. dispar* which is present as an asymptomatic form. He had used a lot of arguments using differential antigen expression as well as the gene which code for those antigens. However, we propose that *E. histolytica* itself could be differentiated into two groups which are found in patients with dysentery and liver abscess and another one is found in asymptomatic cyst passers. We have used a small subunit ribosomal typing to fingerprint such strains into two groups that cause symptomatic and asymptomatic infections. We have also found M D A gene expression in some of symptomatic strains and using a RAPD ribotyping method have been able to further differentiate symptomatic strains from asymptomatic strains. We have immunoscreened cDNA libraries using a Monoclonal agent as 31 KDa Antigen, and developed a probe which is expressed in strains isolated from ALA cases as compared to those obtained from intestinal cases. Hence caution should be exercised in labelling strains from asymptomatic patients as *E. dispar*

S - 6.4

IMMUNOPATHOGENESIS IN GIARDIASIS

V K Vinayak
Dept. of Biotechnology, CGO Complex, Lodi Road, New Delhi

Giardiasis is one of the important public health problems in the country. The prevalence of Giardia lamblia is more common in infants and children and also results in more severe gastro intestinal symptoms in pediatrics population. This is especially so in those who are immune compromised. The adults usually do not have severe symptoms and by and large act as carriers of this infection. The infection by G. Lamblia leads to generation of gul associated immune response and analysis of these responses has been made to associate these responses with the course of the disease in experimental hosts. The regulation of these responses by the parasite surfaces associated molecules has been delinear. On of the glycoprotein for molecules with molecular weight of 56 KDA located on surface membrane of G.lamblia appears to regulate the giardiasis infection. This molecule appeares too have a potential in the eventual development of immunooprophylactic agents for travellers and infants and children.

S-7.1

ANTIFILARIAL AGENTS FROM PLANTS AND THEIR EVALUATION IN ANIMAL MODELS.

Nirmal C Sukul
Department of Zoology, Vishva-Bharati University Santiniketan - 731235, West Bengal

Indigenous plants hold great promise as sources of effective antifilarial agents. This paper describes the preliminary procedures for screening plants for their filaricidal activity, preparation of solvent extracts and testing the extracts *in vitro on Setaria cervi* Effective plant extracts are further analysed in solvents of increasing polarity to isolate and finally identify the active principles. Plant compounds so far found effective are essential oils, particularly monoterpenoids. glycosides, saponins etc. Putative antifilarial agents are tested *in* vivo on *Setaria cervi* transplanted to rats. *Acanthochetlonema vitae. Dirofilaria tmmitis* and *Trichinella spiralis.* Problems and prospects of different animals models are discussed. Effective agents are further tested for their possible side reactions, tolerable and effective doses.

S-7.2

J M Hawdon
(Abstract not received)

S - 7.3

HAEMONCHUS CONTORTUS - THE STOMACH WORM OF RUMINANTS

M L Sood
Department of Zoology, Punjab Agricultural University, Ludhiana - 141 004, India

Haemonchus contortus (Nematoda : Trichostrongylidae) is the most serious parasite occurring in the contents and mucosa of abomassum of sheep/goat. The present review outlines the various aspects of our work on the morphology, biology and metabolism of the adult parasite procured locally from natural infections. Emphasis has been laid on our work on the biochemistry (protein, lipid and carbohydrate), the *in vitro* effects of various anthelmintics on the histopathology and characterization of digestive enzymes and absorption of nutrients in *H. contortus*. The possible future aspects of *Haemonchus* research have been discussed.

S - 7.4

N L Dubey
(Abstract not received)

S - 7.5

S Ray
(Abstract not received)

S - 8.1

WITHIN-HOST DYNAMICS OF *PLASMODIUM FALCIPARUM* AND OTHER *PLASMODIA* IN ASYMPTOMATIC INDIVIDUALS

Marian C Bruce, Christl Donnelly and Karen P Day
Wellcome Trust Centre for the Epidemiology of Infectious Diseases, Department of Zoology, University of Oxford, Oxford, United Kingdom.

In regions with endemic malaria, individuals remain well for the majority of the time despite carriage of parasites by most people. Infections within asymptomatic individuals, therefore, constitute the majority of the parasite population. As such, the dynamics of such infections are of importance for the understanding of the epidemiology of the disease. It has long been recognised that more than one species of *Plasmodia* can simultaneously be present within a host and molecular typing of parasites has shown that infection with more than one genotype of parasite is common in endemic regions. Longitudinal sampling of individuals at 3 day intervals has allowed us to investigate the dynamics of infection with each species. We have investigated the potential effects of the interaction of different species and different genotypes on dynamics of infection in multiply infected people. The results of this investigation into the short-term dynamics of different genotypes of *P. falciparum* within semi-immune children from Papua New Guinea will be presented. How these results may explain epidemiological phenomena observed in cross-sectional surveys will be discussed.

S - 8.2

STRAIN DIVERSITY AND GENETIC EXCHANGE IN MALARIA PARASITES - IMPLICATIONS FOR CONTROL

David Walliker, Lisa Ranford-Cartwright and Hamza Babiker
Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, UK.

While the existence of diverse "strains" of malaria species such as *Plasmodium falciparum* has been suspected for at least fifty years, the full extent of the genetic diversity seen in natural populations of these parasites is only now being revealed. The Polymerase chain reaction (PCR) is being used extensively in many countries to study allelic variants of parasite genes, notably those encoding antigens. A finding common to the majority of studies is that most people in regions highly endemic for *P. falciparum* harbour mixtures of genetically distinct clones. Longitudinal studies have revealed that there may be a considerable turnover of clones from one day to another in some patients, while in others certain clones apparently persist for long periods. The ability of each clones to produce gametocytes clearly influences the extent of crossing between them when they are taken up by mosquitoes, and hence the extent of recombination between genes that will occur. Such considerations have importance with regard to control measures which target the products of more than one parasite gene such as

mixtures of antimalarial drugs or multivalent vaccines.

S - 8.3

IMBALANCED DISTRIBUTION OF *PLASMODIUM FALCIPARUM* MSP-1 ALLELES RELATED TO SICKLE CELL TRAIT

Odile Mercereau-Puijalon[1], Francine Ntoumi[1,3], Christophe Rogier [2], Helene Jouin,[1] Alioune Dieye[2], Pascal Millet[3] and Jean-Francois Trape[4]
1. Unite d' Immunologie Molecular des Parasites, CNRS U1960, Institut Pasteur, Paris.
2. Institut Pasteur, Dakar, Senegal.
3. International Centre for Medical Research, Franceville, Gabon.
4. Laboratoire de Paludologie, ORSTOM, Dakar, Senegal.

Analysis of isolates collected from asymptomatic carriers living in Dielmo, (a holoendemic Senegalese village) indicated that the distribution of the 3 MSP -1 allelic families and of individual MSP-1 alleles was influenced by age and differed in AA and AS subjects. There was no influence of other genetic characters or of location of residence within the village. Distribution of MSP-2 alleles was only marginally affected. In another endemic area with different ethnic groups, transmission intensity and parasite populations, the influence of sickle cell trait on the distribution of parasite types was confirmed in a single age group; a similar imbalance in MSP-1 allele distribution associated with the sickle cell trait was observed in a cohort of Gabonese school children.

The data suggest that the hemoglobin type imposes some constraint on the characteristics of the parasites hosted, reflecting a pressure on MSP-1 itself or on a linked locus. Several, non-mutually exclusive mechanisms could contribute to an imbalanced strain distribution related to host hemoglobin type: selective immune clearance of parasites presenting certain alleles, strain differences in capacity to complete intraerythrocytic development and/or premature elimination of infected cells, resulting from impaired cytoadherence in AA or AS cells. Analysis of the allele specific immune response will be discussed.

S - 8.4

ORGANISATION OF VAR GENES IN POPULATIONS OF *PLASMODIUM FALCIPARUM.*

Chris Newbold, Helen Taylor and Sue Kyes
Molecular Parasitology group, Institute of Molecular Medicine, Oxford, UK.

A number of studies have demonstrated the large degree of genetic diversity in populations of *Plasmodium falciparum* such that it is very rare to find parasites which share alleles at as few as three loci. Similarly, the evidence to date suggests that populations are in general panmictic. Despite the high level of diversity and random mating, recent theoretical predictions have suggested that loci which are important for

parasite survival, such as those involved in eliciting a protective immune response, may be organised within the population by immune pressure. Such a phenomenon could extend to the *var* genes of *Plasmodium falciparum* even though this comprises of large multi-gene family. In order to test this hypothesis we have been developing methods which allow us to compare the entire *var* repertoire of different organisms using a PCR and describe the development of the methodology and its validation using both laboratory clones and recombinants derived from a laboratory cross. Some preliminary data from field isolates will also be presented.

S-8.5

MOLECULAR ASSAYS FOR DRUG RESISTANT MALARIA

Christopher V Plowe
Geographic Medicine, University of Maryland School of Medicine Baltimore, Maryland, USA

Despite much progress in defining the molecular mechanism of *in vitro* resistance to pyrimethamine and sulfadoxine, the determinations of therapeutic failure of these drugs remain unknown. Methods have been developed to detect mutations in the parasite genes encoding antifolate drug targets and used to study the molecular epidemiology of drug resistant malaria in Africa and South America. The prevalence of specific resistance-conferring mutations in *P. falciparum* dihydrofolate reductatse (DHFR) and dihydropteroate system (DHPS) has been correlated with drug availability and usage patterns and *in vivo* resistance levels in different settings. Results from these field-based studies are now guiding efforts to define the molecular and biochemical mechanisms of antifolate resistance. Molecular epidemiologic studies of the antifolate resistance mutations will be summarized and the potential public health utility of molecular assays for drug resistant malaria will be discussed. The status of development of molecular assays for other drugs including chloroquine will also be discussed.

S-8.6

PHYLOGENETIC STUDIES IN THE HUMAN MALARIAL PARASITES : A PROBLEM OF GENES, TREES AND RATES

Ananias A Escalante
Division of Parasitic Diseases, NCID, CDC, U.S., Department of Health and Human Services, Atlanta, GA.

A phylogeny is model that represents the evolutionary history of a group of species. The basic premise supporting any phylogeny is that similarity or shared characteristics are homologous by descent due to a common ancestor. Phylogenetic models usually are inferred using a single gene tree; however, genes evolve differently following their own dynamic. In the case of the phylogeny of malaria parasites, I will present comparisons of three genes : the 18S SSU rRNA, the circumsporozoite protein gene (CSP), and

the cytochrome B from the 6Kb mitochondrial genome. The 18S SSU rRNA genes show some discrepancies when compared with the other two genes, probably because of the inclusion of non-homologous genes into the phylogenetic analysis. However, the major clades are congruent. Phylogenetic analysis based on the 18S SSU rRNA genes should be taken as a preliminary approximation due to their semi-concerted mode of evolution. The CSP gene tree only uses relatively conserved sites in the alignment since faster evolving sites cannot be properly aligned. This fact, in addition to the potential effect of natural selection, limits the CSP use in assessing the overall evolution of the genus. The cytochrome B gene data confirms previous molecular and morphologic observations ab the independent origin of the four human malarial species. *P. reichenowi* is different but closely related to *P. falciparum* and these species clade with the bird parasites. The implications of differences in evolutionary rates among genes will be discussed in terms of detecting horizontal transfer and the potential incrimination of natural reservoirs. In conclusion, gene trees reflect the gene evolution, which may or may not follow the evolutionary history of the species. Genes may evolve at a rate that does not allow them to properly assess a given event. Multi-locus phylogenetic analysis is the best approximation to assess a species' phylogeny.

S-9.1

INNOVATION IN MALARIA DIAGNOSIS

H Kyle Webster
Department of Medical Affairs, Becton Dickinson and Company, Singapore

Prompt diagnosis and appropriate treatment are essential to successful management of malaria infection. Definitive diagnosis is currently based on the proven method of microscopy for detection of malaria parasites in stained blood films. However, quality and availability of thick/thin blood film (TBF) microscopy is highly dependent on expertise of individual microscopists and access to competent microscopy is alarmingly variable even in recognized malarious areas - a situation made increasingly worse by the spread of MDR malaria. Malariologists, tropical and travel medicine specialists, the WHO and National malaria programs have long recognized the values and limitations of TBF microscopy and have sought the development of a comparable less complex and expensive diagnostic test. Innovation in malaria diagnosis has been difficult despite advances in biotechnology. Incremental change has occurred through addition of fluorescent staining but this does not go beyond the restrictions of microscopy for trained individuals and capital costs. Molecular techniques such as PCR and probe hybridization have been successfully used to detect malaria parasites but none have moved beyond the research setting. Presently, the only truly innovative achievement in malaria diagnosis is a rapid manual immunodiagnostic test (dipstick) that uses a monoclonal antibody to detect parasite antigen (pf HRPII) in blood. The new test developed by Becton Dickinson (USA) as a commerical product - ParaSight® F - is comparable in performance to TBF microscopy. Operational field testing now completed in most of the worlds malarious areas has confirmed reliability and ease of the use for ParaSight F. However, traditional barriers to change and perceived cost have slowed widespread adoption - even with the emergence of competitors with a similar (ICT, Australia) or different (Flow, USA) detection system. Perhaps the most difficult aspect of innovation is acceptance. Malaria diagnostic test developers, manufactures and ultimately health care providers must find ways and means to bring new technology to at risk individuals where and when it is needed.

S - 9.2

M T Makler
(Abstract not received)

S - 9.3

M Garcia
(Abstract not received)

S -9.4

FIELD EXPERIENCE WITH PARASIGHT F

Joseph B Perrone
Becton Dickinson Microbiology Systems, Becton Dickinson Co. Sparks, Maryland, USA

A review of recent field trials using ParaSight F will be presented. Studies from Venezuela. Thailand and Brazil will be summarized. Additional comments will be made on patients' perceptions of this test and improved clinic attendance due to the use of this diagnostic tool. Information will also be presented to address the controversy surrounding the question of antigen persistence of HRP-II. Earlier theories proposed that patients with ParaSight F positive tests and negative blood films after an apparent successful course of therapy was due to antigen persistence. It now apears that many of these apparent false positive are due to sequestered parasites not detectable by blood smears. The potential for ParaSight F to be used as an indicator of treatment failure will also be discussed.

S - 9.5

S Ray
(Abstract not received)

S - 9.6

CHALLENGES IN MALARIA DIAGNOSIS

Wilbur K Milhous and Dennis E. Kyle
Walter Reed Army Institute of Research, Washington, DC, USA

There is an urgent and serious need for alternatives to microscopy for malaria diagnosis at all levels of the global health care system. In endemic areas where malaria is normally part of the differential diagnosis and where microscopists are trained and have adequate equipment, this poses few problems. With the increased prevalence of drug resistant strains, increased international travel and commerce along with changes in ecology and human demographics, there are more and more patients with unusual disease presentations in a variety of health care settings. Often malaria is not considered in the differential diagnosis, and the results can be tragic. With the development of the new rapid and innovative diagnostic tests such as Parasight F and Optimal, significant advances have been achieved. These tests differ in terms of sensitivity and specificity as well as adaptability to patient management in contrast to initial diagnosis. The focus this discussion will be to evaluate new methods to diagnosis malaria and to examine the role for these new technologies in routine malaria diagnosis and for patient management with special emphasis on strategies for aligning the goals of obtaining regulatory approval with global markets.

S - 10.1

UNDERSTANDING THE IMMUNE RESPONSE TO MALARIA IN MOUSE MODELS : RELEVANCE FOR HUMAN INFECTIONS.

Jean Langhorne, Mattias Berg, Caroline Cross and Elsa Seixas
Department of Biology, Imperial College of Science, Technology and Medicine, London SW7 2 BB, United Kingdom.

Experimental models of malaria have been used to determine mechanisms of protective immunity and immunopathology for all stages of the life cycle of the malaria parasite. Despite the fact that rodent parasites are not natural pathogens of the laboratory mouse, the general principles underlying immune responses to the extracellular and intracellular stages are likely to be similar in rodents and humans. Therefore, it can be expected that the mechanisms elucidated in the various models can be applied to large extent to the immune response in humans. For example, as found in human infections, an expansion of $\gamma\delta$ T cells in infected mice is restricted to cells with a limited TCR repertoire and which produce predominantly IFN-γ. Experiments with knock -out mice deficient in $\alpha\beta$ or $\gamma\delta$ T cells indicate a limited role for $\gamma\delta$ cells in the early control of blood stage parasites. Their role in pathology is not yet certain.

Studies in all rodent models have demonstrated an essential role for CD4+ T cells in the protective immune response to blood stage parasites. However, the contribution of the different effector arms of the CD4+ T cell response differs in the different models. Some parasites are more readily controlled in the absence of antibody whereas others require antibody responses. Rather than contradictions, these data should be seen as representing the spectrum of effector arms available for elimination of blood stage parasites. Experiments in a variety of gene-targeted mice have allowed us to dissect the important events (cytokines and cells) important in regulations of CD4+ T cells responses. Since these cells play a crucial role in immunity to malaria, an understanding of the control of their responses would be very valuable for the design of vaccines and for the understanding pathogenesis.

S - 10.2

T V Rajan
(Abstract not received)

S - 10.3

THE MAJOR SURFACE PROTEIN 1 (MSP 1) OF PLASMODIA IS A TARGET OF PROTECTIVE IMMUNITY AGAINST PREERYTHROCYTIC AND ASEXUAL STAGE MALARIA PARASITES.

Sanjai Kumar[1], Sylvia I Becker[1], Brennan R Harding[1], Ruobing Wang[1], Malcolm J Gardner[1], David C Kaslow[2], and Stephen L Hoffman[1]
1. Malaria Program, Naval Medical Research Institute.
2. The Laboratory of Parasitic Diseases, NIH, Bethesda, MD, USA

The major surface protein1 (MSP1) of plasmodia is a high molecular weight protein, expressed on merozoites of liver stage and blood stage parasites. Previous vaccination studies with purified recombinant protein containing the 19-KD C-terminal, epidermal growth factor-like domain of MSP1, in complete Freund's adjuvant (CFA) induced protection against *Plasmodium yoelii* (PyMSP1) in mice and *Plasmodium falciparum* (PfMSP1) in monkeys. The protective formulation with CFA is not suitable for use in humans. Having established that immune responses against the MSP1 C-terminus can be protective in mice and monkeys, we are assessing other strategies for immunogen development. One strategy is to produce DNA vaccines. We have constructed a number of plasmids including Py or Pf MSP1 sequences. In the *P. yoelii* model, immunization of mice with a plasmid encoding the C-terminus or with a mixture of the C-terminus plasmid and a plasmid encoding the NH2-terminus induced significant protection against sporozoite challenge; protection which was comparable to that induced by administration of a C-terminus purified recombinant protein in CFA. Interestingly, the DNA vaccine constructs did not induce protection against challenge with infected erythrocytes comparable to the protection found after administration of the recombinant protein. This raised the possibility that the PyMSP1 DNA vaccines induced protective immunity against the liver stage of the parasite life cycle. PfMSP1 C-terminal and N-terminal DNA vaccines have been shown to induce murine antibodies that recognize liver stage and asexual erythrocytic stage Pf parasites, and are currently being assessed in Aotus monkeys.

S - 10.4

THE MACAQUE MODELS OF HUMAN MALARIAS : THEIR USE FOR EVALUATION OF VACCINES

K N Mendis[1], K L R L Perera[1], S M Handunnetti[1] and S Longacre[1]
1. Malaria Research Unit, Faculty of Medicine, University of Colombo, Sri Lanka.
2. United' Immunoparasitologie, Institut Pasteur, Paris, France.

Macaques, old world monkeys indigenous to South East (SE) Asia are natural hosts of several species of simian malaria parasites. They include *Macaca mulatta* (the rhesus) prevalent in much of SE Asia and *Macaca sinica* (the toque monkey) indigenous to Sri Lanka and South India. Of the malaria parasites naturally infecting these macaques,

some species are considered to be analogous to human malaria parasites, and in their natural hosts, constitute good models for the study of human malarias. We have used two such natural parasites of *Macaca sinica, Plasmodium cynomolgi* and *P. fragile* as analogues of *P. vivax* and *P. falciparum* malaria respectively in humans based on similarities in morphological and genomic level between the simian and human parasites.

These natural host-parasite pairs have often been overlooked in preference to artificial systems in which the human parasite has been adapted to unnatural hosts (nonhuman primates). Unnatural host-parasite systems tend to produce highly virulent infections, which are very unlike the naturally adapted host parasite systems of humans or monkeys, and may lack relevance to the human infection. We have used two natural parasites of the toque monkey *P. cynomolgi* and *P. fragile* as models of human *P. vivax* and *P. falciparum* respectively for immunological studies, in particular for evaluating the potential of a vaccine candidate of *P. vivax* in humans.

S - 10.5

SELECTIVE EXPANSION OF α β T CELL POPULATION DURING MURINE CEREBRAL MALARIA

D Mazier, Sylviane Pied, M Idrissa-Boubou, D Voegtle*, P A Cazenave*
INSERM U 313, CHU Pitie-Salpetriere, 75013 Paris.
* CNRS URA 1961, Institut Pasteur, 75724 Paris cedex 15.

In order to characterize T cells involved in the pathogenesis of cerebral malaria in murine models, we first demonstrated, using α β $TCR^{-/-}$ and γδ $TCR^{-/-}$ C 57 BL/6 mice, the importance of α β T cells in the genesis of the disease. Second, we analyzed the repertoire of TCR Vβ segments usage for T cells from blood, lymph nodes and spleen of mice infected with *P. berghei* ANKA. Analysis were done by flow cytometry using monoclonal antibodies directed against the different Vβ families.

Data show that cerebral complication in C57 BL/10. D2 mice infected with *P. berghei* ANKA are associated with an increase of $V\beta8^+$ $CD3^+$ T cells in the blood. This increase was very pronounced in mice manifesting profound neurological syndromes. The link between these T cell populations and neuropathology is strengthened by the observation that congenic Balb. D2 Mls 1^a mice, which display a peripheral deletion of T cells expressing the Vβ8.1 segment, do not develop cerebral malaria while being susceptible to the infection. The mechanisms whereby these $V\beta8^+$ $CD3^+$ lymphocytes are involved in the immunopathological process are under investigation.

S - 11.1

NEOGLYCOPROTEIN CONJUGATED MURAMYL DIPEPTIDE IN THE THERAPY OF VISCERAL LEISHMANIASIS

Pijush K Das
Molecular Cell Biology Division, Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Jadavpur, Calcutta-700032.

Active targeting of muramyl dipeptide (MDP) to macrophages was studied by conjugation with the neoglycoprotein, mannosyl human serum albumin (Mannose-HSA) using visceral leishmaniasis as the model macrophage disease. Conjugation did not decrease the affinity of the neoglycoprotein for macrophage mannose receptor. Mannose-HSA-MDP was 50 times more efficient than free MDP in inhibting the growth of *Leishmania donovani* inside peritoneal macrophages. Moreover, in a 60-day murine model visceral leishmaniasis, 95% of spleen parasite burden was reduced by mannose-HSA-MDP at a dose of 0.5 mg/kg/day given for 4 days. Free MDP at a similar dose had very little effect. In vitro exposure of MDP caused enhanced generation of O_2 by macrophages wherease generation of NO ws not induced. The elevated antileishmanial tivity of MDP-treated macrophages in culture was abrogated by O_2 scavengers. In contrast, considerably enhanceamounts of NO and O_2 was generated from macrophages of mannose-HSA-MDP treated animals, and their splenocytes secreted soluble factors providing all the signals required for the induction of No biosynthesis. Increase in NO production was paralleled by a concomitant increase in antileishmanial activity which was reversed by NO synthesis inhibitors. Splenocyte supernatants treated with anti-IFN-γ or anti-TNF-α antibodies suppressed inducible NO generation by macrophages. Moreover, intravenous administration of anti-IFN-γ and anti-TNF-α along with mannose-HSA-MDP greatly reduced protection against *L. donovani* infection. Neoglycoprotein-conjugated MDP, therefore, activated mouse macrophages in vivo to kill *L. donovani* and this may depend on the physiological operation of NO induced by IFN-γ and TNF-α.

S - 11.2

P Kumara Swamy
(Abstract not received)

S - 11.3

DIFFERENTIAL IMMUNE RESPONSE IN BANCROFTIAN FILARIAL PATIENTS OF WHOLE PARASITIC AND RECOMBINANT FILARIAL ANTIGEN

P Kaliraj
Centre for Biotechnology, Anne University, Madras 600 025, India

Bancroftian filariasis in humans is caused by the nematode parasite, *Wuchereria bancrofti*, is a disease of major public health problem in tropical countries like India. The clinical

groups of filariasis patients includes (i) Asymptomatic microfilaraemics with circulating microfilaria (MF), (ii) Patients with chronic pathology (CP) and (iii) Endemic normal (EN) who are asymptomatic and amicrofilaraemic. Among the various groups, the MF individuals have lymphocyte hyporesponsiveness toward whole parasitic antigen. Further the hyporesponsiveness seen in MF has been studied at the gene level by reverse transcriptase polymerase chain reaction (RT-PCR). In this study, we have demonstrated the role of IL-10 in hyporesponsiveness in MF patients when compared to CP and EN. For the purpose of developing the vaccine strategies for filariasis, humoral and cellular immune responses of the filarial patients to either the whole parasitic (*Brugia malayi* antigen) or purified recombinant filarial antigen (pGT51 were studied. All the categories of the individuals (EN, MF and CP) examined showed the presence of circulating antibodies against both whole parasitic and recombinant filarial antigens but at different levels. MF individuals exhibited higher levels of the IgG4 sub class antibodies. A differential cellular response was seen at different stages of infection. While the proliferative responses were normal with all the individuals examined for mitogens, the lymphocytes from MF individuals showed significantly lower proliferative response to whole parasitic antigens, when compared to the other clinical groups (EN and CP). In contrast, the recombinant antigen elicited normal proliferative response even from MF individuals. These results clearly indicate that the initial cellular energy is possibly down-regulated by some components in the total mixture of antigens. Identification of the parasite antigen factor(s) responsible for effecting Th1 response should prove valuable in vaccine development.

S-11.4

STUDIES ON EXPERIMENTAL *WUCHERERIA BANCROFTI* INFECTION IN INDIAN LEAF MONKEY *(PRESBYTIS ENTELLUS)*

R K Chatterjee
Division of parasitology, Central Drug Research Institute, Lucknow - 226 001, India.

Lymphatic filariasis is a major tropical disease affecting millions of people in India and elsewhere in the tropical world. In India *Wuchereria bancrofti* is the major species of filaria which claims more than 90 percent of lymphatic filaria which claims more than 90 percent of lymphatic filarial cases. Nonavailability of target parasite (W. bancrofti) is suitable laboratory host has seriously jeopardised sfforts on development of drugs and our understanding on mechanism of disease process which is believed to be immune mediated. Several attempts made in the past on experimental, transmission of *W. bancrogfti* infection in rodents and cats has so far been unsuccessful. Studies recently made in our laboratory has led to establishment of *W. bancrogti* in Indian leaf mondey (*Presbytis entellus* commonly known as langur. The mostr favoured seat of predilection of adults was inguinal lymph glands followed by popliteal glands and testes. Diethylcarbamazine showed lethal effect on adult parasites. Infected monkeys developed pathological changes with enlargement of lymph glands and dilatation of lypmphatics etc. IgG and IgM antibody responses were detected in sera as early as 20

days following infective exposure. Most of the exposed animals responsiveness to mitogen.

Indian leaf monkey thus appears to be a suitable nonhuman primate host for *Wuchereria bancrofti* studying the host parasite interactions and for teriary screening of potential antifilarid.

S - 11.5
M V R Reddy
(Abstract not received)

S - 11.6

CLINICAL EPIDEMIOLOGY OF LYMPHATIC *FILARIASIS* : AN UPDATE
S P Pani
Vector Control Research Centre, Medical Complex, Indira Nagar, Pondicherry - 605 006, India

Lymphatic filariasis disease presents a spectrum of manifestations, which range from allergic conditions like TPE, acute episodic Adenolymphangitis (ADL), hydrocele and lymphoedema. Understanding the mechanisms of disease development is absolutely essential for the development of morbidity control strategies. The current control programme is for transmission control by vector control of selective chemotherapy. The need for morbidity control for prevention of progression of disease is not addressed properly. In the recent past, there has been progress in our understanding of clinical epidemiology of filariasis. Definition of the disease status has been formulated clearly. It has been recognized that most of the microfilaraemics who are asymptomatic by routine clinical examination have lymphatic abnormalities, predisposing them for development of disease. Acute episodic attacks of ADL have been recognized for their social and economic impact on individuals and their families. It has been recognized that development of hydrocele and lymphoedema follow different patho-mechanisms. The dynamics of infection and disease appears to be modulated by mf carriers who are mostly asymptomatic while TH! proinflammatory response is observed in chronic patients who are usually amicrofilaraemic. The mechanism of this transition of one form of immunity to the other inducing disease is still not understood. Increasing evidence of bacterial pathogens particularly group A *streptococcus* in predisposing ADL attacks and increased frequency of these attacks with progression of lymphoedema have opened the possibility of new morbidity control tools. However there are areas which still require attention, for example, the long term efforts of DEC in infection and disease dynamics is not known. These new findings and future possibilities will be discussed.

S - 11.7
S K Kar
(Abstract not received)

S - 12.1

INTRACELLULAR DELIVERY OF DRUGS THROUGH RECEPTOR-MEDIATED ENDOCYTOSIS

Sandip K Basu
National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi-110 067.

Toxic side effects resulting from the administration of therapeutic agents often complicate successful therapy in a number of diseases. The adverse side reactions possibly arise due to the fact that at therapeutically effective concentrations in the blood, the non-target cells in the body are also exposed to the cytotoxic effects of the drugs. It is probable that such side effects could be minimized if a modality of delivering drugs could be worked out which would : (i) reduce the uptake of the drugs by non-target cells, (ii) selectively deliver the drug only to the target cells at relatively low concentration in the blood, and (iii) ensure efficient intracellular availability of the drug. Current approach to site-specific drug delivery consists of attaching the therapeutic agent to a determinant unit recognized only by the cells where pharmacological action is desired. Two types of recognition elements on the surface of target cells are being exploited for this purpose viz., (i) antigens capable of generating specific, non-cross reactive antibodies, and (ii) receptors on the cell surface capable of efficient transport of the ligands. In general, incomplete specificity for the target cells and poor internalization of antibody-drug conjugates limit the usefulness of antibodies for site-specific drug delivery applications. We have been exploring the alternate possibility of exploiting the exquisite cell type specificity and high efficiency of endocytosis of macromolecules mediated by specific receptors present on the surface of some mammalian cells types. As macrophages are affected in a large number of viral, bacterial, fungal, protozoal, metabolic and neoplastic diseases, we have demonstrated the feasibility of this approach for delivering drugs selective to these cells. Our approach consists of chemical coupling of an appropriate drug to a carrier molecule, *e.g.* maleylated albumin which is recognized by specific receptors (called scavenger receptors) present exclusively on cells of macrophage lineage. We have shown that such drug conjugates bind with high affinity to the scavenger receptors on macrophage surface leading to rapid internalization and subsequent degradation of the ligand in lysosomes releasing a pharmacologically active form of the drug. So far we have tested the efficacy of this approach in cell culture and animal models of macrophage-associated disorders of protozoal (leishmaniasis), bacterial (tuberculosis) and neoplastic etiology. In all three instances examined, the conjugated drug was nearly 100-fold as effective as the free drug. These results indicate that the receptor-mediated modality of delivering drugs to macrophages could contribute to greater therapeutic efficacy and minimization of toxic side effects in the management of intracellular infections as well as neoplastic diseases. We have also recently demonstrated (in collaboration with S. Rath and V. Bal) that targeting of antigens as well as for modifying the type of immune response generated , thus providing a new and general strategy for increasing the efficacy of vaccines. Maleylated albumin-mediated delivery of various agents, therefore, provides a generalized tool for manipulating the metabolic

activity of macrophages for a variety of purposes.

S - 12.2

A NOVEL PEPTIDE-GRAFTED LIPOSOMAL DELIVERY SYSTEM TARGETED TO MACROPHAGES

M K Basu, Goutam Banerjee and Swapna Medda
Biomembrane Division Indian Institute of Chemical Biology 4, Raja S C Mullick Road Calcutta 700 032, India

Interaction of chemotactic peptide e.g. f-Met-Leu-Phe grafted liposomes with the macrophages is noted to be rapid and specific. At 100 nmoles concentration of grafted peptide, the internalization of the peptide grafted liposomes by the macrophages is found to reach equilibrium in 30 mins. The peptide alone and the peptide-grafted empty liposomes are found to show moderate antileishmanial activity *in vitro*. Primaquine which is known to generate O_2^- in phagocytic cells (e.g. macrophages) showed much better efficacy in artificial leishmaniasis when used in the f-Met-Leu-Phe grafted liposomal form in comparison to either free or encapsulated in ungrafted liposomes. Interestingly, it is non-toxic to macrophages for a certain range of concentration tested *in vitro*. The conventional toxin parameters e.g. blood pathology, tissue histology, specific enzymes level related to normal liver function are found to be very close to those of the normal for the f-Met-Lue-Phe grafted liposomal primaquine. The biodegradability of both the drug and the delivery systems are also found to be very satisfactory. Thus this delivery system may have possible applications in leishmaniasis, as well as in other macrophages associated disorders.

S - 12.3

NEOGLYCOPROTEIN CONJUGATED MURAMYL DIPEPTIDE IN THE THERAPY OF VISCERAL LEISHMANIASIS

Pijush K Das
Molecular Cell Biology Division, Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Jadavpur, Calcutta-700032.

Active targeting of muramyl dipeptide (MDP) to macrophages was studied by conjugation with the neoglycoprotein, mannosyl human serum albumin (Mannose-HSA) using visceral leishmaniasis as the model macrophage disease. Conjugation did not decrease the affinity of the neoglycoprotein for macrophage mannose receptor. Mannose-HSA-MDP was 50 times more efficient than free MDP in inhibting the growth of *Leishmania donovani* inside peritoneal macrophages. Moreover, in a 60-day murine model visceral leishmaniasis, 95% of spleen parasite burden was reduced by mannose-HSA-MDP at a dose of 0.5 mg/kg/day given for 4 days. Free MDP at a similar dose had very little effect. In vitro exposure of MDP caused enhanced generation of O_2 by macrophages wherease generation of NO ws not induced. The elevated antileishmanial tivity of MDP-

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S - 12.4

EFFECT OF VARIOUS LIPOSOMAL AND LIPID FORMULATION OF POLYENE ANTIBIOTICS IN THE TREATMENT OF EXPERIMENTAL *ASPERGILLOSIS*

Prahlad C Ghosh, Sandeep Saxena, and Junaid A Khan
Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi-110 021.

Aspergillosis, a fungal disease caused by *Aspergillus fumigatus,* is common among immunosuppressed patients such as persons suffering from tuberculosis, cancer, AIDS, etc. Fungizone, a commercial preparation of amphotericin-B (amp-B) in deoxycholate, is the only accepted therapy to control such systemic fungal infection. However, its well known toxicities have limited its clinical application. Lipid formulation of amphotericin-B has been used to improve the treatment of a variety of experimental and human infection. However, the optimal lipid formulation for amphotericin-B delivery has not been established, but lipid composition greatly affects efficacy and toxicity.

In the present studies, we used a mice model of invasive aspergillosis to compare the efficacy and toxicity of polyene antibiotics (amphotericin-B and hamycin) in various liposomal formulations (conventional and stealth) and in a colloidal dispersion of cholesterol sulfate. The toxicities of amphotericin-B and hamycin was reduced by 5-6 fold when they were administered in colloidal dispersion form. Similarly, liposomal drugs (SPC : Chol) also showed significant reduction in toxicity as compared to free drugs. Preliminary results showed that the toxicities of these polyene antibiotics is reduced by 2-3 fold when administered through stealth liposomal formulation. It was also observed that the therapeutic efficacy of these polyene antibiotics is highly dependent on the lipid formulation used for their delivery. Results of these studies will be discussed.

S - 12.5

C M Gupta
(Abstract not received)

S - 12.6

NOVEL GENE DELIVERY TO LIVER CELLS BOTH *IN VITRO* AND *IN VIVO* USING ENGINEERED VIROSOMES

Debi P Sarkar
Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021 India.

We have demonstrated for the first time that the reconstituted Sendai viral envelopes containing only the fusion protein (F-virosomes) are efficient vehicles for the delivery of foreign genes specifically into human hepatoblastoma cells (HepG2) in culture. The membrane fusion mediated entry of CAT (chloramphenicol acetyI transferase) gene into the cells was confirmed and the amount delivered to various subcellular fractions was quantitation. The dose-dependence and kinetics of expression of biologically active CAT protein in HepG2 cells was measured. The CAT expression level in F-virosome-mediated delivery was significaly higher than that of Lipofectin or liganded proteo-liposome-mediated gene transfer. The efficiency of F-virosomes-mediated targeted gene delivery to mouse liver cells has also been demonstrated *in vivo*. This kind to targeted delivery by means of membrane fusion induced by viral envelope glycoprotein may have wide applications to various gene transfer strategies both *in vitro* and *in vivo*.

S - 13.1

THE BIOLOGY OF MALARIA TRANSMISSION TO THE MOSQUITO VECTOR : ITS PROMOTION AND BLOCKADE BY ANTIMALRIAL COMPOUNDS

R E Sinden, G A Butcher, A Gamage-Mendis and B Hogh
Department of Biology, Imperial College, London, National Institute of Health, Maputo, Mozambique and Statens Serum Institute, Copenhagen, Denmark.

Infectivity of gametocyte carriers to the mosquito is modulated by a wide range of factors. These individual factor originate from the parasite, the vector and the vertebrate host, and from interactions between these 3 organisms.

Using laboratory studies on P. berghei and field studies on P. falciparum we have examined the impact of two commonly used antimalrials, chloroquine and Fansidar, upon the infectivity of parasites to the mosquito vector.

Both drugs block the development of mature gametocytes fromhe asexual stages. Mature gametocytes are insensitive to both compounds. In P. falciparum gametocyte carriers results in a declining population of viable sexual stages over the 21 days following treatment. Fansidar blocks the infectivity of the persisting mature gametocytes to the mosquito very effectively.

In contrast chloroquine promotes the infectivity of mature gametocytes by as much as 4 fold, this enhancement is highest 21 days after treatment of the patient. In addition these is evidence that following chloroquine treatment, chloroquine resistant parasites produce more gametocytes (4.4 fold higher) than sensitive lines. Taken together this data may explain both the failure of chloroquine to suppress malaria transmission in endmic areas, and the rapid spread of chloroquine resistance. The data would suggest it might be wise to reappraise regimens of chloroquine administration.

S - 13.2

TOWARDS MOLECULAR MECHANISMS OF GAMETOCYTOGENESIS IN *PLASMODIUM FALCIPARUM*

Nirbhay Kumar, Cheryl Lobo and Kunyan Zhang
Department of Molecular Microbiology and Immunology, John Hopkins University School of Public Health, Baltimore, MD, USA

The unicellular protozoan parasites belonging to the genus *Plasmodium* have a complex life cycle in which the asexual multiplication of parasites in the vertebrate host alternates with an obligate sexual reproduction in the mosquito vector. Erythrocytic sexual stages (male and female gametocytes) are produced by sexual differentiation of asexual stages within the vertebrate host and are responsible for transmitting the parasite to the mosquito vector. The sexual reproduction of the parasites in the mosquitoes involves a

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complex set of events leading to the formation of extracellular female and motile male gametes, which undergo fertilization and further development. It is during the mating between male and female gametes that genetic recombination occurs during meiotic division of fertilized zygotes, and is also believed to be a major factor contributing to enormous genetic diversity of the parasite population. Such diverse parasites are then disseminated into the human population during further transmission. Sexual differentiation and development of the malaria parasite thus occupies a central place in the biology of the parasite that poses a major health risk worldwide.

The initiation of sexual differentiation in *P. falciparum* occurs early after invasion of erythrocytes by sexually committed merozoites. A clonal population of haploid parasite can generate both male and female gametocytes, Sexually differentiated parasites undergo profound morphological, biochemical and functional changes. This is accompanied by preferential expression of a number of proteins in a coordinated fashion during distinct maturation stages of gametocytes defined as Stages I to V. Pfg 27 (Mr = 27,000) is one such cytoplasmic protein expressed at the onset of gametocyte differentiation and the expression continues throughout gametocyte maturation. However, the molecular mechanisms involved in the differentiation of sexual stages of malaria parasites are unknown. We are using an approach based on subtraction hybridization between unidirectional cDNA expression libraries of the asexual and sexual stages to identify genes differentially expressed in the sexual stages.

Recently we have also investigated the role of protein phosphorylation and dephosphorylation as potential mechanisms during sexual differentiation of *P. falciparum*. Modification of proteins by phosphorylation is a reversible process catalyzed by kinases and phosphatases and represents mechanisms by which cells respond to extracellular signals, mechanisms of signal transduction and regulation of cell cycle, etc. A number of proteins expressed in the asexually replicating stages of malaria parasites have been shown to be modified by protein phosphorylation. The asexually replicating stages of malaria parasites have been shown to be modified by protein phosphorylation. The question of protein phosphorylation in the sexually differentiated stages has not been addressed, and it was hypothesized that unique protein phosphorylation events might play significant role in the overall process of sexual differentiation. We observed that Pfg27 expressed in the sexual stages is phosphorylated predominantly at the threonine residues. This is the first report on the identification of a sexual stage-specific protein phosphorylation in *P. falciparum*.

S - 13.3

ROLE OF TRAP IN INFECTIVITY OF MALARIA SPOROZOITES

Robert Menard[1] Ali A Sultan[1], Vandana Thathy[1], Ute Frevert[1], Kathryn J Robson[2], Andrea Crisanti[2], Victor Nussenzweig[1] and Ruth S Nussenzqeig[1]

1. New York University Medical Centre, New York, New York 10016
2. Institute of Molecular Medicine, Oxford, OX3 9DU, UK

3. Imperial College of Science, London, SW7 2BB, UK

Plasmodium sporozoites need to invade the secretory cells of the mosquito salivary glands and hepatocytes of the mammalian host to induce malaria infection. Like other invasive stages of Apicomplexan protozoans, Plasmodium sporozoites exhibit gliding motility *in vitro*, a form of substrate-dependent cell locomotion characterized by the absence of locomotory organelles. This active locomotion of sporozoites, which is correlated with their infectivity, is thought to account for their ability to reach and enter the target cell. Plasmodium sporozoites express a surface protein called thrombospondin-related anonymous protein (TRAP), also named sporozoite surface protein 2 (SSP-2), that is conserved in all plasmodial species and has homologues in other Apicomplexa. TRAP is a typical single - pass transmembrane protein which contains two matrix-or cell - binding regions and a highly conserved cytoplasmic tail. TRAP binds to sulfated glycoconjugates associated with the hepatocyte plasma membrane, and antibodies raised against TRAP inhibit sporozoite invasion of hepatocytes *in vitro*.

Using the gene targeting technology in the rodent parasite *P. berghei*, we have disrupted the single - copy TRAP gene. *P. berghei* TRAP(-) erythrocytic stages normally invade and develop in erythrocytes. In mosquitoes, TRAP, which is expressed in midgut sporozoites, is important for sporozoite invasion of the salivary glands. In the mammalian host, TRAP is required for sporozoite infection of the liver. TRAP is also essential for sporozoite gliding motility *in vitro*.

These results suggest that gliding motility and cell invasion in Plasmodium, and likely in other Apicomplexa, are mechanistically related. Both events depend upon the microfilament system of the parasite and are thought to result from the capping the substrate - or cell - parasite surface complexes from the anterior to the posterior pole of the parasite. It is conceivable that TRAP links the parasite microfilament system to the substrate during gliding locomotion and to the target cell surface during invasion.

S - 13.4

CONSEQUENCES OF STAGE SPECIFIC EXPRESSION OF THE rRNA GENES OF PLASMODIUM BERGHEI IN WILD TYPE AND TRANSFECTED PARASITES

A P Waters, J Thompson, R E Sinden, R M L van Spaendonk & C J Janse.

The expression of the stage specific rRNA genes of rodent malaria parasite, Plasmodium berghei, has been studied using northern analysis and in situ hybridisation to both bloodstage and mosquito stage parasites. The analysis reveals a highly regulated pattern of gene expression regulated at the level of transcription. A mechanism of ribosome replacement has been revealed and periods where multiple ribosome forms are active observed. Mutant parasites which have had specific rRNA gene units disrupted have been produced and their characterisation will be presented.

S - 13.5

PLASMODIUM VIVAX GENES EXPRESSED IN MEROZOITES DEFINE TWO NEW PROTEIN FAMILIES IN MALARIA PARASITES

John W Barnwell, Mary R Galinski, Basima Al-Khedery, German Rosas-Acosta, Louis H Miller, Diane Hudson-Taylor and Mengyao Xu and Paul Ingravallo
New York University School of Medicine, Department of Medical & Molecular Parasitology, New York, NY,USA
Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD, 20892, USA

The surface coat of *Plasmodium vivax* to date consists of at least five different proteins designated in numerical order of discovery as PvMSP-1, PvMSP-2, PvMSP-3, PvMSP-4, and PvMSP-5. The last three MSPs (3 to 5) constituted an inter-species gene family. The three proteins share only 30% identity between their amino acid sequences but very similar structurally. Each protein has a signal peptide sequence, N-terminal and C-terminal acidic hydrophilic domains and a large central core domain that is enriched in alanine content, which is strongly predicted to form multiple coiled-coil tertiary structures. The extent of diversity within this family between isolates of *P. vivax* suggests they are targets of naturally acquired immune responses. Similarities in structure and amino acid alignments at the amino and carboxy ends of the proteins indicate that this family includes members in *P. falciparum* and *P. knowlesi.*

The Reticulocyte Binding Proteins (RBP-1 and RBP-2) form a receptor protein complex that had an important biological role in targeting reticulocytes for invasion by *P. vivax* merozoites. These two proteins also have counterpart family members in *P. yoelii, P. falciparum* and *P. knowlesi.* PvRBP-2 is related to the 235 kDa rhoptry protein family of *P. yoelii*, which has numerous genes in the genome of this parasite. We have also isolated two separate genes from *P.falciparum* which share homology to PvRBP-2. Cross reactive antibodies also suggest that a protein similar to PvRBP-1 is also expressed in *P.falciparum. P. knowlesi* also has genes that encode proteins that are highly homologous to the RBPs of *P. vivax*. The RBPs of *P.vivax* bind to reticulocytes whereas the *P.yoelii* 235 kDa rhoptry proteins bind to and mediate invasion of normocytic erythrocytes. Similarly, both *P.falciparum* and *P.knowlesi* largely invade normocytes and are not restricted to reticulocytes. This suggests that the members of this protein receptor family have a wide, more general, functional role in merozoite invasion than first suggested by the reticulocyte-specific binding of the *P.vivax* RBP receptor complex.

S - 13.6

ANALYSIS OF A MOLECULAR INTERACTION INVOLVED IN ERYTHROCYTE INVASION BY *PLASMODIUM* MEROZOITES

Chetan Chitnis, Akash Ranjan, Sanjay Singh and Kailash Pandey

Malaria Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India.

The invasion of erythrocytes by *Plasmodium* merozoites is a multi-step process that is mediated by specific molecular interactions. For example, the human malaria parasite *P. vivax* and the related simian parasite *P. knowlesi* require interaction with the Duffy blood group antigen and invasion of human erythrocytes. *P. knowlesi* also invades rhesus erythrocytes by Duffy antigen independent pathways. *P. falciparum* does not use the Duffy antigen for invasion but most strains bind sialic acid residues of glycophorin A for invasion. The parasite proteins that bind these receptors share similar features and belong to a family of erythrocyte binding proteins that includes the Duffy binding protein of *P. vivax* and *P. knowlesi* and the sialic acid binding protein of *P. falciparum. The* extracellular domain of each of these proteins contains two cysteine-rich regions in which the cysteines and a number of hydrophobic amino acid residues are conserved. Erythrocyte binding assays using recombinant domains expressed in mammalian cells showed that the functional erythrocyte binding domain of each protein lies in the 5' cysteine-rich region. The binding domains contains approximately 350 amino acids with 12 to 14 cysteines. Chimeric domains containing parts from the *P. vivax* and *P. knowlesi* binding domains were expressed mammalian cells and to used map the binding site of the *P. vivax* Duffy binding protein. An approximately 100 amino acid stretch between the fourth and seventh cyteines was found to contain the binding site. This stretch will be targeted for site directed mutagenesis to identify the contact residues. The site on the Duffy antigen that *P. vivax* binds to has also been mapped. A 35 amino acid peptide from the N-terminal, extracellular region of the Duffy antigen could block the binding of *P. vivax* domain to erythrocytes indicating that it is the site used for binding. As we begin to understand the structural basis of these receptor-ligand interactions that are crucial for invasion, it may be possible to design or screen for molecules that can inhibit these interactions and block erythrocyte invasion. It has already been shown that chemokines such as IL-8 and MGSA, which bind the Duffy antigen, can block the invasion of human erythrocytes by *P. knowlesi* highlighting the feasibility of such a strategy.

S - 13.7

HOST UROKINASE-TYPE PLASMINOGEN ACTIVATOR PARTICIPATES IN THE RELEASE OF MALARIA MEROZOITES FROM INFECTED ERYTHROCYTES

Catherine Braun-Breton, Emmanuelle Roggwiller, Anne-Catherine Fricaud and Thierry Blisnick
Unit of Experimental Parasitology, URA CNRS 146, Department of Immunology, Institut Pasteur Paris, France

Plasminogen activators are serine proteases which process the plasma proenzyme plas-

minogen into active plasmin, a serine protease with broad specificity, involved in extracellular matrix degradation. Cell surface binding of uPA to a specific, glycolipid-anchored receptor can generate functionally active plasmin at the cell surface and seems to be involved in the process of tissue invasion by tumor cells. Plasminogen activation also being associated with pathogenic bacteria, is involved in the degradation of the host extracellular matrix and seems to be a prerequisite for bacterial invasion of host tissues.

The association of plasminogen activation with invasive properties of malaria parasites has been investigated. We have detected plasminogen activators in *Plasmodium chabaudi* and *Plasmodium falciparum* extracts and identified these enzymes as host urokinase-type plasminogen activator. The binding of the circulating host enzyme to a specific receptor at the surface of malaria infected erythrocytes seems to participate in the process whereby malaria merozoites are released from red blood cells.

The putative substrate for urokinase and its receptor at the surface of malaria-infected erythrocytes are potential targets for specific antibodies or inhibitors affecting the intraerythrocytic cycle of the parasite without affecting urokinase activity itself. Their identification and characterization are in progress.

S - 13.8

CHARACTERISTICS OF THE IMPORT OF RED CELL δ–AMINOLEVULINATE DEHYDRASE INTO THE MALARIAL PARASITE

G Padmanaban and Zahid Q Bonday
Department of Biochemistry, India Institute of Science, Bangalore : 560 012, India.

The Malarial parasite has acquired a unique mechanism to carry out heme biosynthesis *de novo*. It only codes for the first enzyme of the pathway namely δ-aminolevulinate(ALA) systhase. On the other hand, it imports ALA dehydrase and perhaps the other enzymes of the pathway from the host. Red Cell ALA dehydrase with histidine tag was expressed in *E.coli* and its binding characteristics to othe parasite membrane were studied. [^{35}S]-Methionine labeled ALAD binds specifically to the parasite (*P.berghei*) membrane as revealed by radioactivity measurement and immunogold electron microscopy and the binding can be completely chased by excess cold enzyme. The dissociation constant and the number of binding site have been estimated to be 5.16 x 10^{-9} and 1.6 x 10^{3} respectively. Further His-tagged ALAD was incubated with solubilized parasite membrane and then reisolated using a nickel-agarose column. SDS-Page analysis of the enzyme eluted from the column reveals an additional 35 Kda band in addition to that of the His-tagged ALAD subunit (~40da). Analysis of appropriate controls does not reveal the additional band. It is likely that the 35Kda protein in the parasite membrane is involved in the specific binding of red cell ALAD.The mechanism of translocation of this protein into the parasite is of considerable interest

S - 13.9

ANSWERING MALARIA QUESTIONS IN TOXOPLASMA GONII : DRUG RESISTANCE MECHANISMS AND PARASITE CELL BIOLOGY

David S Roos, Mary Reynolds, Sabine and Robert G.K. Donald
Department of Biology, Univ. Pennsylvania, Philadelphia PA 19104 USA

Among the > 5000 known parasites of the phylum apicomplexa, Toxoplasma godii is particularly well-suited to laboratory study - providing the opportunity to explore many questions of relevance to malaria in a more experimentally tractable system. Asexual tachyzoites (merozoites) are easily cultivated in vitro, permitting automated screens for parasiticidal compunds. Superb ultrastructural resolution facilitates a wide variety of cell biological studies. Sexual crosses are feasible (in cats), permitting classical genetic analysis. Lacking the A/T bias observed in many Plasmodium species, recombinant T. gondii genes are readily amenable to manipulation and expression in heterologous systems. Molecular genetic tools permit transient and stable transformation, using integrating or episomal vectors, and both high - and low-copy number selectable markers. The molecular genetics of T. gondii is distinguished by two remarkable features : (i) frequencies of stable molecular transformation are features : (i) fequencies of stable molecular transformation are sufficiently high for complementation cloning (>1%), and (ii) genomic integration can be accomplished by either homologous or non-homologous recombination (depending on the length of contiguous genomic DNA introduced). Homologous recombination allows targeted gene knock - outs and allelic replacements, while high-frqquency nonhomologous recombination permits insertional mutagenesis - the entire parasite genome can be tagged in a single experiment, and tagged loci are readily cloned by plasmid-rescue techniques. Taking advantage of the accessibility of this system, it is possible to address many questions of interest of the malaria.

S - 14.1

SOCIAL AND BEHAVIOURAL ASPECTS OF MALARIA CONTROL

Halima Mwenesi
TDR / IDRC I TNS Initiative Kenya Medical Research Institute P O Box - 20752
Nairobi, Kenya

Most treatment for malaria occurs outside of the formal health sector, where it is first defined and diagnosed. Since about half the population in the developing world has no access to public health services, home management of childhood malaria is inevitable. We examined carer's definition/recognition of the disease and pathways of care seeking for childhood malaria on the kenyan coast. Malaria is defined as a self-limiting natural disease, with recognizable symptoms including fever, joint pains and lethargy. Complications associated clinically with malaria such as convulsions are not recognized as such. Treatment for the condition includes home remedies, over-the-counter (OTC) drugs and as a last resort the official health sector.

While self-medication indicates prompt presumptive treatment, inappropriate use of the OTCs is a serious cause of concern. Retail-outlet proprietors and other drug vendors have little knowledge of the drugs they sell and most carers are unable to visually identify the antimalarials they mention as treatment for childhood malaria.

Further, it is not only OTC drugs that are used inappropriately. Even medications prescribed in health facilities are used in the same manner, partly because no clear instructions accompany the prescribed drugs or because carer's use wrong measures when administering the drugs. Policy implications of these findings are discussed.

S - 14.2

MALARIA TRANSMISSION, VECTOR CONTROL AND PERSONAL PROTECTION

Umberto D' Alessandro
Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium.

Recent studies on the impact of insecticide-treated bednets (ITN) have shown a considerable impact on childhood mortality ranging from 17% to 33%. However, concern on long-term efficacy of an ITN programme on child mortality has been raised by several authors. These are based on the observation that variations of malaria-specific mortality and severe disease are weak compared to the considerable range of transmission levels and that only a considerable reduction of transmission will have a long-term effect on the burden of malaria. A careful examination of the evidence reveals important problems. Data from Brazzaville indeed show that extreme differences in malaria transmission may be associated with only minor differences in severe malaria incidence rates. However, it should be noted that these were much lower than those reported from

other African regions, the widespread use of antimalarials and the higher socioeconomic status have been suggested as possible reasons, and are clearly unrepresentative of most of sub-Saharan Africa. Data on the relation between malaria mortality rates and intensity of transmission were reviewed recently by Snow and Marsh. Malaria mortality rates were determined by using verbal autopsies and the unreliability of this approach has been stressed already. In some cases, information on the causes of death was collected several years after the event, making the diagnosis even more unreliable. Another factor of unreliability is the use of different methods to estimate the intensity of transmission. There is the urgent need to establish the relation between intensity of transmission and malaria mortality and particularly to evaluate the long term effects of ITN. The evidence accumulated so far, is not sufficient to warrant abandoning the implementation of ITN programmes.

S - 14.3

DRUG RESISTANCE IN MALARIA - EVOLUTION AND PRESENT STATUS IN INDIA.

S P Misra,
Dte. National Malaria Eradication Programme, 22, Sham Nath Marg, Delhi 110 054.

Early diagnosis and prompt treatment is a strategy by itself for malaria control and the Directorate of National Malaria Eradication Programme has been regularly and systematically monitoring the susceptibility of *P. falciparum* to antimalarials with WHO recommended procedure since 1978. Studies have revealed that there is wide variation in the proportion of treatment failures (RII & RIII responses) within a district and the responses are not always consistent within a Block/Primary health centre. In the country chloroquine resistant *P. falciparum* (CRPF) is steadily increasing in degree and frequency. Treatment failure was observed to have significantly increased ($P<0.001$) from 4% initially to 13.6% in 1990-1994. There was statistically significant difference in the prevalence of CRPF among the various regions in 1978-1984 but by 1994 the proportion of treatment failures with chloroquine became similar except in North-Central region.

In developmental project areas there was rapid increase in treatment failure and at any given period resistance was significantly higher ($p<0.01$) in the project areas as compared to non-project areas.

Treatment failure with Sulfa-pyrimethamine is low (2.1%) and has been observed mainly in the north-eastern States. Amodiaquine offers no advantage over chloroquine as cross resistance is parallel and complete.

The differential rate of development and spread of chloroquine resistance in the various regions is apparently associated with the intensity of Pf transmission and degree of population movement. In areas with minimal population movement and intense Pf

transmission evolution of resistance has been tardy. The distribution and evolution of resistance to chloroquine that it is multifocal in origin with gradually changing sensitivity to chloroquine on a locally selective basis. Apparently the rate of development and spread of CRPF is relatively slow as compared to other countries.

S - 14.4

INSECTICIDE-IMPREGNATED BED NETS : FROM EFFICACY TRIALS TO SUSTAINABLE PROGRAMMES

J Cattani and C Lengeler

Insecticide-treated nets proved very effective in reducing child mortality in a non-randomized trial carried out in The Gambia in 1989-1990. All cause mortality in the age group 1-4 years using bed nets treated with permethrin at the dose of 500 mg/m^2 was reduced by 63 per cent compared to a control population. This important finding led to a major initiative by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) designed to determine whether these findings could be replicated in randomized controlled trials in a 4 different epidemiological setting in Africa (Burkina Faso, The Gambia, Ghana and Kenya). Nearly 400.000 people were included in the four trials of which three investigated the impact of treated bednets and one (in Burkina Faso) investigated the impact of treated windows, eaves and door curtains. Reductions in all-cause mortality in children aged 1-59 months ranged from 15 to 33 percent. The results have confirmed the significant impact that treated nets can have on child mortality in Africa and supports their widespread introduction as a part of effective malaria control.

Operational research is currently in progress on improving the technology involved in impregnation and re-impregnation of nets as well as on issues related to cost-effectiveness and social and economic factors affecting the implementation and promotion of insecticide-treated nets in Africa. Access, affordability and sustainability, whether nets and/or insecticide are made more widely available through the private market or provided at subsidized prices or with full cost recovery through donors or the public sector (or in mixed models with a combination of private/public sector participants), are the three key issues that will determine the success or failure of treated nets as an effective intervention over time. Evaluation of the long-term impact of insecticide-treated nets in malaria control in Africa should also be accorded a high priority.

S - 14.5

B Sharp

(Abstract not received)

S - 14.6

EPIDEMIC PREPAREDNESS AND RESPONSE FOR MALARIA CONTROL IN INDIA

Shiv Lal
National Malaria Eradication Programme, 22-Sham Nath Marg, Delhi - 110 054, India.

Malaria has been a serious public health problem in India and the control efforts were initiated way back in 1901 with thrust on source reduction and anti-larval operation. Till the advent of residual insecticide DDT, control efforts were directed towards aquatic stages of vector in the treatment of malaria cases. In 1953, an organised National Malaria Control Programme (NMPC) was launched in India in highly endemic areas with residual insecticidal spraying for transmission control as the major strategic approach. A highly significant decline in malariometric indices could be recorded within the first five years of its implementation and encouraged with the result an ambitious country-wide National Malaria Eradication Programme (NMEP) was launched in 1958. The programme began with insecticidal spraying and was strengthened with the introduction of active surveillances. Dramatic results could be achieved by 1965 when the pre independent estimates of morbidity of 75 million cases annually became 0.1 million cases of malaria during the year. There was no death due to malaria as against the pre independence estimates of 8 lakh deaths annually. The resurgence of malaria was recorded in 70s which reached all time high pick of 6.47 million cases in 1976. The proportion of *P. falciparum* malaria also increased with the record of 0.84 million cases. A revised strategy was implemented as Modified Plan of Operation (MPO) with subsequent integration through Primary Health Care System. With the changing epidemiological pattern of malaria as a consequence of ecological changes, newer paradigm have been recognised and the area specific strategies are being implemented in the programme. The enhanced malaria control programme is also being introduced for malaria control in tribal and hardcore areas with a thrust on more reliance on community involvement and inter-sectoral co-ordination. The programme has been facing severe technical constraints like increasing problem of drug resistance among the parasite, insecticidal resistance among the vectors, increasing malariogenic potential due to developmental activities, urbanisation, migration related to increased communication facilities , etc. The intensity and severity of malaria outbreak have increased in recent past in India and outbreaks have been recorded in various parts of the country like Assam, Nagaland, Rajasthan, Haryana, Maharashtra, etc. in last three years. To meet this challenge, a specific component of epidemic preparedness and response has been introduced in the programme. The thrust areas that include prediction on the basis of vulnerability and potential of an area by incorporating various factors like endemicity, meteorological indices, receptivity, etc. Early detection through an effective surveillance and monitoring system at the grassroot level and the district level and effective response through an organised system with adequate logistics support to minimise the time between occurrence and containment, are discussed in this paper.

S - 14.7

RESPIRATORY DISTRESS COMPLICATING SEVERE MALARIA IN AFRICAN CHILDREN : EPIDEMIOLOGICAL AND CLINICAL IMPLICATIONS.

Mike English
Dept Paediatrics, University of Oxford, JRH

Recent work in Kilifi, coastal Kenya, an area of endemic *P. falciparum* transmission demonstrates that most malaria deaths occur in the context of simple, clinical syndromes. These syndromes, neurological impairment, respiratory distress (RD) and severe anaemia are individually associated with case fatality rates of 7.3%, 14% and 1.3% respectively, with rates rising dramatically in overlap groups (mortality 28% if both neurological impairment and RD are present). RD is closely associated with the presence of an underlying metabolic acidosis which in several areas of sub-Saharan Africa and in Papua New Guinea is the single most important predictor of death in severe malaria. This strong association means a single clinical sign, deep (acidotic breathing, might adequately represent the syndrome of RD. This sign might allow primary healthcare workers to identify children who require urgent inpatient management and allow possible geographical differences in the presentation of severe malaria to be examined. However, poor agreement on the definition of this sign may currently be limiting its usefulness clinically and epidemiologically.

The aetiology of RD/acidosis in malaria remains poorly understood but both anaemia and dehydration may play important roles in pathogenesis while salicylate toxicity may also contribute in some children. Whatever the cause rapid access to blood and intravenous fluids for resuscitation is essential to immediate management. Whether access to more effective antimalarial therapy early in the course of clinical malaria would prevent this complication or whether the problem of diagnostic overlap with acute respiratory infection can be overcome remain important questions.

S - 14.8

MALARIA DURING PREGNANCY IN TRIBAL BELT OF CENTRAL INDIA.

Neeru Singh[1] and V.P. Sharma[2]
1. Malaria Research Centre (Field Station), Medical College Building, Jabalpur - 482003
2. Malaria Research Centre, 20, Madhuvan, Delhi

It is generally agreed that pregnant women are especially prone to severe attacks of malaria which may cause abortion, premature labour and still birth. Only few hospital based studies carried out in different parts of India provides limited information. Therefore, This study was undertaken (1996-1997) in tribal belt of Central India, an area of perennial malaria to elucidate the prevalence of malaria in pregnant women to define some of the effects that it may exert during pregnancy to provide information for the development of malaria control strategy for this high risk group.

In all 137 pregnant women and 140 non pregnant women were selected randomly in 10 tribal villages of Narayanganj PHC of Distt, Mandla during active transmission period and followed them longitudinally for the duration of pregnancy and puerperium. Slide positivity rate was over 70% in pregnant women and 50% in non pregnant women. *Plassmodium falciparum* was the most prevalent species accounting for 90% of total malaria infection in both pregnant and non pregnant women. There were no instances of cerebral malaria or death, however abortion's, still birth and Anaemia was commonly prevalent in most of the women (63%). Malaria parasites were not found in 25 neonates born to mothers with heavy parasitaemia at the time of delivery. However, two neonate were found positive for *P. falciparum* on Day 10 and Day 18 after birth.

Failure to clear *P.falciparum* parasitaemia after chloroquine administration (25mg/kg of body weight) was common. Persistent parasitaemia was recorded in 30% pregnant women while only in 19% of non pregnant women ($p < 0.001$). Poor response to chloroquine suggests the need to change the drug policy.**S - 14**

S - 14 .9

O Walker
(Abstract not received)

S - 14.10

THE EFFECTS OF MALARIA AND MALARIA PREVENTION IN PREGNANT WOMEN RESIDING IN A HIGHLY MALARIA ENDEMIC SETTING IN SUB-SAHARAN AFRICA

R Steketee
Centers for Disease Control and Prevention, Atlanta, GA, USA.

The control of malaria in pregnant African women is one of several child survival strategies applied through antenatal care that has been particularly challenging. Prevention and control recommendations for typical areas of high *Plasmodium falciparum* transmission promoted the use of antimalarial chemoprophylaxis to prevent placental infection. Persistently low program coverage coupled with diminishing intervention effectiveness have forced a re-evalution of the relative importance of malaria in pregnancy. The Mangochi Malaria Research Project (MMRP), a prospective evaluation of malaria prevention in pregnant women in rural Malawi conducted during 1987-1990, contributed to establishing new criteria for policy and program development for malaria prevention in pregnancy. The principal findings of the MMRP include: 1) populations at risk of the adverse consequences of malaria in pregnancy include women with low parity, HIV-infection pregnancy during the high malaria transmission season, and the use of a suboptimally efficacious antimalarial drug. 2) the estimated maximum benefits of an antimalarial intervention that clears placental and umbilical cord parasitemia

are a 5%-12% reduction of all low birth weight, approximately 35% reduction of low birth weight which is preventable once a woman is pregnant, and a 3%-5% reduction of infant mortality. 3) the intervention must be capable of renderning the woman malaria parasite free, including clearance of parasites from the placental vascular space and umbilical cord blood. 4) other diseases adversely effect pregnancy outcome and while the control of malaria in pregnancy may not warrant independent programming, if coupled with prevention programs to provide a range of antenatal services, the incremental cost of malaria control may prove to be highly cost effective and 5) the choice of a regimen must balance intervention efficacy with safety, availability, affordability and simplicity of delivery, and several antimalarials may meet these criteria. The Malawi Ministry of Health has modified its malaria prevention in pregnancy recommendation and now faces the challenges of effective programming to improve child survival.

S - 14.11

K Patel
(Abstract not received)

S - 14.12

THE USE OF IRON SUPPLEMENTATION AND/OR MALARIA CHEMOPROPHYLAXIS FOR THE PREVENTION OF SEVERE ANAEMIA AND MALARIA IN AFRICAN CHILDREN EXPOSED TO INTENSE *P.FALCIPARUM* TRANSMISSION

C Menendez[1], E Kahigwa[2], R Hirt [1,3], Vounatsou[4], J J Aponte[1], F Font[1,3], J Kimario[3], B Brabin[5], M Tanner [4] and P L Alonso[1]

1. Unidad de Epidemiologia Bioestadistica, Hospital Clinic, Barcelona, Spain.
2. St. Francis Designated District Hospital, Ifakara, Tanzania.
3. Ifakara Centre, (National Institute for Medical Research), Ifakara, Tanzania.
4. Dept. of Epidemiology and Public Health, Swiss Tropical Institute, Basle.
5. Liverpool School of Tropical Medicine, UK.

Iron deficiency anaemia and malaria are two leading causes of morbidity and mortality among infants and young children world-wide. *P. falciparum* infection contributes to the aetiology and severity of anaemia through several mechanisms. Routine iron supplementation is often recommended, but there is some conflicting *in vivo* and *in vitro* evidence on the effects of iron deficiency or overload in determining the severity of infectious diseases in man. Several studies raise the possibility of increased susceptibility to malaria in individuals receiving iron. We report the results of a four-arm, randomised double-blind placebo-controlled trial of iron and/or malaria chemoprophylaxis in the prevention of anemia and malaria among infants exposed to intense and perennial malaria transmission.

832 infants were individually randomised to receive either daily oral iron (2 mg/kg/day) plus weekly Deltaprim® (3.215 mg pyrimethamine plus 25 mg dapsone), or iron pius weekly placebo, or daily placebo plus weekly Deltaprim® or daily placebo plus weekly placebo. Daily supplementation was given from 8 to 24 weeks of age, while the weekly intervention was given from 8 to 48 weeks of age. The incidence of severe anaemia [packed cell volume (PCV)<25%] and malaria episodes was assessed through passive case detection.

Iron supplementation was associated with a reduction of 28.8% (95% CI:6.3%, 45.8%) in the risk of severe anaemia whereas it had no significant effect on the risk of malaria episodes [PE = 12.8% (95% CI: -12.8%, 32.5%)]. Malaria chemoprophylaxis reduced the risk of severe anaemia by 57.3% (95% CI: 43.0%, 67.9%) and prevented 60.5%(95% CI: 48.2%, 69.9%) of first or only episodes of malaria. However, after completing the interventions, children who had been on malaria chemoprophylaxis had an increased risk of having an episode of severe anaemia [RR=2.2 (95% CI: 1.3, 3.7)], or malaria[RR=1.8(95% CI 1.3, 2.6)].

Malaria chemoprophylaxis appears to have impaired the development of naturally acquired immunity. Iron supplementation has been efficacious at preventing severe anaemia without increasing susceptibility to malaria. The results of this study support the large scale provision of iron for the prevention of iron-deficiency anaemia in infants, including those living in malaria endemic areas.

S - 16.1

CULTIVATION OF *PLASMODIUM VIVAX* USING A METHOD THAT ALTERNATES BETWEEN A STATIC AND SHAKER CULTURE

Claudia F Golenda
Department of Entomology, Walter Reed Army Institute of Research, Washington, DC, 20307-5100, USA

A cultivation method was developed in which *Plasmodium vivax* parasites (Chesson strain) obtained from owl and monkeys were grown to schizogony in McCoy's 5A medium modified with L-glutamine with 25 mM Hepes buffer supplemented with 20% AB^+ human serum. At schizogony, parasites were passaged to human reticulocytes and recycled in human cells every other day for 7 parasite generations. Reticulocytes were enriched by differential centrifugation in homologous plasma from the blood of a hemochromatosis patient. Parasites were grown during each 48 hr cycle in a static candle jar environment until the beginning of schizogony, at about 36-40 hr, when reticulocytes were added and cultures were transferred to a shaker for 10-12 hr. The addition of a concentration of 10% reticulocytes resulted in stabilizing parasite densities between 0.28-0.57 after cycle 3 and increasing the total number of parasites at least 2-fold with each generational cycle. Cultures were reinitiated from cultured parasites that were cryopreserved in glycerol and stored for 9 months at - 70°C. Approximately 20-30% of the rings and young trophozoites recovered and matured to schizonts. However, at the beginning of schizogony (37 hr), cultures contained a high density of erythrocyte micelles which interfered with recycling. Although rings were observed at 50 hr, the density had decreased. Research is currently focussed on improving recovery and recycling of cryopreserved parasites and cultivation of other strains of *P. vivax*.

S - 16.2

J le Bras
(Abstract not received)

S - 16.3

EXOERYTHROCYTIC (EE) DEVELOPMENT OF *PLASMODIUM GALLINACEUM* (*Pg*) SPOROZOITES, A MALARIA PARASITE OF FOWL, AND EFFECTS OF ANTISPOROZOITE ANTIBODIES

Antoniana U Krettli, Eliana MM Rocha and Adelina D Ramirez
Centro de Pesquisas Rene Rachou/FLOCRUZ Belo Horizonte, MG, Brazil

Avian malaria, extensively used in the past, represents an excellent model for malaria studies especially *Pg*, considered to be phylogenetically related to the human parasite *P. falciparum* (*Pf*). Currently it is accepted that : (a) sporozoites enter and reach full devel-

opment in macrophages and in endothelial cells in avian malaria; and in hepatocytes in mammalian malaria; (b) the initial EE schizogony releases merozoites which reinvade other tissue cells, only in the case of avian malaria; (c) the late relapses of some mammalian species including *P. vivax* , result from subpopulations of sporozoites which remain quiescent in hepatocytes for months or years. Aspects of the EE-host interactions with antibodies to sporozoites, of *Pg* known to be protective *in vivo*, were studied. Macrophages cultivated from chicken bone marrow cells, as well as endothelial cells from a chicken cell line (SL-29 from AATC) support sporozoite invasion (6% and 0.5%, respectively) and full development of the EE cycle at 37°C, 5% Co_2. Within 70h mature schizonts with free EE merozoites are observed. However, the EE merozoites forms did not initiate secondary EE cycle, i.e., reinvasion was not observed. These EE forms are highly infective to chicken : they caused intense parasitism of the endothelial cells lining the brain capillaries; parasitaemias ≤ 30% and death within 2 weeks. Specific anti-*Pg* sporozoite mAb's showed variable degrees of *in vitro* inhibition of parasite invasion (ISI) in macrophages and SL-29 cells. A straight correlation between *in vitro* and *in vivo* activity of mAb's was observed. Thus, a complete inhibition of the EE cycle was induced by mAb's which *in vivo caused* total neutralization sporozoites infectivity . We proposed that *Pf* sporozoites may be taken up and destroyed by monocytes and/or Kuppfer cells *in vivo* in the presence of antibodies from immunized and protected volunteers, even before hepatocytes.

S - 16.4

IN VITRO CULTIVATION OF ERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM*

Virendra K Bhasin
Department of Zoology, University of Delhi, Delhi 110 007, India

Continuous cultivation of erythrocytic stages of *P. falciparum* has made it possible for research workers all over the world to have the clinically important phase of the malarial life cycle *in vitro*. Various culture media and procedures which have wide acceptance and are being used in many laboratories for culturing *P. falciparum* will be described. Importance of some of the factors required for cultivation and scaling up the parasite yield will be discussed. Some of the cofactors essential for intraerythrocytic development have been identified. Perhaps one of the most important aspect of cultivation is that it places the organism under nearly controlled conditions. This control is greatly increased if the cultures are grown in a defined medium and under axenic (extracellular) conditions. Work toward this goal has progressed to the point where it has been possible to obtain *in vitro* extracellular development of the entire schizogonic cycle from merozoites. This occurred in a complex medium based on and erythrocyte sonicate and Matrigel. Unfortunately, this method can not be used for continuous axenic cultivation because at best only 2 to 3% of the merozoites complete the cycle, even though 20 to 30% transform into rings. The work has shown a role for red cell cytoskeleton in the maturation of rings to trophozoites. The axenic development occurs

in the absence of a parasitophorous vacuole membrane. Further work should help to clarify the parasite-host relationships of such obligate intracellular parasites as malaria and other Apicomplexa.

S - 16.5

MALARIA TRANSMISSION BLOCKING IMMUNITY

Richard Carter
University of Edinburgh, Edinburgh, UK

At any stage in the life cycle of malaria, immunity which reduces the infectivity of a parasitized host to the mosquito vectors is a form of malaria transmission blocking immunity (TXI). When the term was first applied, however, TXI specifically concerned immunity against the mosquito infecting sexual stages of the parasites. It has become general practice to use the term "transmission blocking immunity" principally in relation to immune effects which directly prevent entry of the parasites into, or their successful development in the mosquito. In this presentation I will review the variety of mechanisms and effects that could be considered to fall within this definition of malaria TXI. These include effects innate to the host or to the state of malarial infection, inducible immune effects against the parasites as they enter or develop within the mosquito vector, and inducible immune effects against specific products of the mosquito which are necessary for survival of the parasites in the vector. The prospects of each as the basis of future malaria transmission blocking vaccines will be briefly discussed. In the second part of the presentation I will analyse the likely impacts of applying transmission blocking vaccination, or indeed any malaria inoculation reducing measures, under different endemic situations.

S - 16.6

IN VITRO CULTIVATION OF TISSUE STAGES OF MALARIA PARASITE

Kamal K Kamboj
Division of Parasitology, Central Drug Research Institute, Lucknow-226001. India

The tissue stage of malaria parasite has often been described as "hidden" or the "silent" forms in the life cycle of the malaria parasite. These forms remained a mystery for many years and were discovered as late as 1948. Even after their discovery, they remained inaccessible to researchers because of their location in a deeper organ, the liver. These were also earlier thought to be free from host immune attack.

The discovery that human hepatoma cell line (HepG2) supports invasion of malaria sporozoites and their intracellular development greatly facilitated research on these "hidden" stages. Further, the development of methods for the isolation of viable hepatocytes

by enzymatic perfusion and their successful maintenance in primary cultures have allowed studies on these forms in the homologous host cells.

The in vitro systems can be suitably employed to act as primary screen systems for evaluating the efficacy of potential tissue schizontocidals, candidate vaccines, cytokines, etc. .

S - 17.1

V Southgate
(Abstract not received)

S - 17.2

TREMATODE INFECTIONS OF LIVESTOCK IN NORTH-EAST INDIA

V Tandon
Department of Zoology, North-Eastern Hill University, Shillong 793 022, India

The livestock (cattle, buffalo, goat, sheep and pig) are found to have a high prevalence of trematode infections in the north-eastern states of India viz., Assam, Meghalaya, Mizoram, Nagaland and Tripura. The ruminants slaughtered in Assam and Tripura and pigs in all the states under study represent mainly an indigenous stock. The trematode parasite spectrum in these animals comprises at least 31 species. The host-wise species composition in the spectrum and prevalence of infection in the various regions has been well documented. Host animals from a low-to-medium range of altitude show a high prevalence, whereas those from the high altitude zones have a relatively low rate of infection. Though amphistomes constitute the most prominent component of infection, different fluke species predominate in different host groups and regions. At least 3 amphistomid parasites, 2 in Meghalaya and 1 in Tripura, represent the species not reported hitherto from other regions of the country. The surface fine topographical features of most of these species provide newer authentic tools for their taxonomic identification.

Prevalence of *Fasciola gigantica* and *Eurytrema pancreaticum* in cattle and *Fasciolopsis buski, Artyfechinostomum malayamum, Gastrodiscoides hominis* and *Opisthorchis noverca* in pigs bears a zoonotic significance in the region. The biology of these parasites (which implicates snails intermediate host in their life cycle) in conjunction with different physiographical and ambient climatic conditions needs to be studied for evaluating their transmission dynamics and epidemiological characteristics.

S - 17.3

PARAGONIMUS AND PARAGONIMIASIS IN MANIPUR.

T Shantikumar Singh
Department of Microbiology, Regional Institute of Medical Sciences Imphal, Manipur, India.

During the recent years paragonimiasis has become one of the important public health problems in Manipur. Up to now about 300 patients have diagnosed and treated for paragonimiasis at the Regional Institute of Medical Sciences and Hospital alone in Imphal, Manipur. Clinical and laboratory analysis of 150 cases revealed pulmonary /

pleuropulmonary paragonimiasis as the commonest form followed by cutaneous, intra abdominal, pericardial and cerebral paragonimiasis. Ingestion of encysted metacercariae in raw or inadequately cooked fresh water crabs or crabs juice constituted major mode transmission of infection. Infrequently, application of minced raw crabs on the diseased skin for a cure is another possible mode of transmission. *Potamiscus manipurensis* and civet cats served as important second intermediate host and final animal host in nature, respectively. More than two *Paragonimus* species are prevalent in Manipur. In Manipur as in Thailand and China *paragonimus heterotremus* is considered medically important species. A definitive diagnosis can be made by finding *Paragonimus* ova in sputum, faeces, and rarly in the aspirated pleural fluid or subcutaneous cystic malt and positive skin test for paragonimiasiss. A 3-day-therapy with praziquantel given orally in dosage of 25 mg per kg body weight 3 times a day is recommended as it provided complete cure in all the patients who received this regimen.

S - 17.4

H Sugiyama
(Abstract not received)

S - 17.5

ANIMAL SCHISTOSOMES AND THEIR ZOONOSES IN INDIA

M C Agrawal
Department of Parasitology, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh.

A number of avian and mammalian schistosome species were discovered in India some of which need confirmation using modern techniques. Widely prevalent and more known mammalian schistosomes are-*Schistosoma indican, S. spindale. S. nasale, S. incognitum* and *Orientobilharzia dattai,* all of which may occur in an endemic area, In animals, schistosomiasis is occurring in two forms-nasal and hepatic. Though the former is caused by *S.nasale* and occurring only in bovines, yet it has been considered more widespread than hepatic form. This is erroneous since *I.exustus* not only spreads *S.nasale* but also *S.indicum* and *S.spindale* (Causative agents of hepatic form) which have more wide host range. This wrong perception developed because of easy diagnosis of nasal form (by snoring sound) and difficulty in diagnosing hepatic form as faecal egg detection has poor sensitivity.

The possibility of human schistosomiasis in India was excluded by not finding suitable snail hosts for known human schistosomes. However, discovery of endemic focus of urinary schistosomiasis, in Gimvi Village of Ratnagiri District with *Ferrissia tenuis* as its intermediate host has removed this complacency. Moreover, possibility of Gimvi

Fluke being a *S. indicum* variant, first demonstration of *S. incognitum* eggs in human stools presence of *S.nasale* flukes in lung and liver of goats with absence of their eggs in faeces and nasal cavity, absence of a systematic survey for human schistosomiasis and poor sensitivity of diagnostic survey for human schistosomiasis and poor sensitivity of diagnostic tests being employed in India, strongly suggest human sufferings because of Indian schistosomes. This should be confirmed by utilizing modern diagnostic tests like detection of "circulating antigens" which can differentiate among cercarial dermatitis, graded latent schistosome infections, mature infections either with or without passing the eggs in human faeces.

S - 17.6

TREMATODE INFECTION OF PUBLIC HEALTH IMPORTANCE IN ASSAM

J Mahanta
Regional Medical Research Centre, N.E. Region (ICMR), Dibrugarh, Assam, India.

Public health importance of trematode infection has been underestimated in many countries. Recently there is a renewed focus on this group of parasites because of its considerable impact on the health and economics in terms of morbidity, loss of productivity, agricultural losses, etc. Earlier intestinal trematodes of public health importance were mostly reported from certain South East Asian and far east countries. However, our experience in North East region show that food borne trematode infections and paddy field dermatitis are of serious public health concern in this region. At least seven intestinal trematodes have been found to cause human infection in North Eastern region of which *Fascioplopsis buski, Gastrodiscoides hominis, Paragonimus,* and *Paryphostomum sufrartyfex* have been found earlier in Assam. However, recently we have identified foci of Heterophyesis, Clonorchiasis and Fascioliasis for the first time from this region, out of which *Fasciola hepatica* and *Clonorchis sinensis* cause considerable morbidity in man. The clinical, pathological and epidemiological features of these and other trematode infections in Assam are discussed. In addition to the above trematode parasites, animal schistosomes have been incriminated as a cause of paddy field dermatitis in Assam. It is a serious occupational disease and at least 41,000 agricultural labourers in Upper Assam are at risk. Vectors, animal pathogenicity and pathology in experimental animals due to Schistosomiasis have been worked out.

S - 18.1

FIELD RESEARCH FOR MALARIA CONTROL : SOME LESSONS LEARNED AND SOME SUGGESTIONS FOR THE FUTURE

Louis Molineaux
Peney-dessus, Satigny, Geneva 1242, Switzerland

An epidemiologist's bird's eye view of past and current field research for malaria control : broad types of (1) the questions addressed, (2) research methodologies (study designs and measurements (3) actors (investigators and sponsors) (4) relationships between field research and control. Some of the related current problems and some suggestions for the future.

S - 18.2

BIOTECHNOLOGY OF PARASITIC DISEASES - NETWORK PROGRAMME

Manju Sharma
Department of Biotechnology Government of India, II block, 7th Floor, CGO Complex Lodi State, New Delhi

Parasitic diseases world over are the cause of high morbidity and mortality, particularly in the tropical and sub-tropical world. Research and development on parasitic diseases is therefore, one of the thrust areas identified to formulate new approaches for control. Modern biomedical research with the application of biotechnological tools have already resulted in the development of diagnostics, vaccines, and pharmaceutical products, and a new global market has been created.

The Department of Biotechnology, Government of India has created infrastructure for research and development, and established centres of human resources development in many nationally relevant areas of biomedical research. Special emphasis has been given on diseases like Malaria, Filariasis, Leishmaniasis and Intestinal parasitic Diseases. Multi-centric and interactive programmes have been generated involving molecular characterization, genome sequencing, identification of genes for diagnostics, modelling, therapeutics, prophylactics, drug delivery systems and host-parasite interactions. Network programme on a particular disease involving research institutions, industries and other related agencies has become a very essential and effective institutional framework. It is largely the indisciplinary and networking approach which has given rich dividends in terms of product, processes, technologies and strategies for reducing the burden of parasitic diseases. Salient features of these and related developments would be presented.

S - 18.3

A Kitua
(Abstract not received)

S - 18.4

THE MALARIA FOUNDATION IN THE 90'S

Mary R Galinski
Washington square Village, Suite 10F New York - 10012.

The mission of The Malaria Foundation is to facilitate the development and implementation of solutions to the health, economic and social problems caused by malaria. To this end, the Malaria Foundation's current goals are 1) to assist with the development and coordination of networks that enhance communications, maximize exchange of views and expertise among malaria scientists and health workers, and encourage the most cost-effective use of available resources, 2) to educated individuals and institutions about the enormity of the malaria problem worldwide, and help to secure financial assistance for malaria research and control programs, and 3) to support the education and training of our next generation of malaria experts. These goals have been rigorously formulated in the 90's as we have looked to see where immediate needs were and where we as a new organization could make a difference. These areas, otherwise summarized as networking, public awareness, and training, will remain our main focus for the remainder of this decade.

The development of the Malaria Foundation as an independent international nonprofit organization has been a "grass roots" efforts and developed from the very generous contributions of numerous committed individuals who have been willing to listen, generate ideas, act (i.e. volunteer their services), publicize the foundation's efforts, gather supporters, and take a stand for its mission and goals. The good will of people worldwide is what has made the Malaria Foundation come to exist and it will also be the basis for the most productive global networking efforts in malaria research and control that lie ahead. We take this opportunity to recognized the contributions of the people, scientists and other professionals, as well as representatives of many organizations including the NIH, the EC, USAID, WHO, CDC, the BWF, RAM*, and industries who have stood behind the Malaria Foundation and provided a boost to its growth and development.

S - 18.5

THE MALARIA RESEARCH NETWORK

Carole Long, John Barnwell, Dyan Wirth, Mary Galinski, Chris Sokol and Robert Nagel
Department of Microbiology and Immunology, Allegheny University of the Health Sciences, 2900 Queen Lane Philadelphia, PA 19129

The scope and nature of the problems posed by malaria have highlighted the need for greater communication and collaboration in this field. As a result, in coordination with the Malaria Foundation, we have developed the Malaria Research Network (MRN) to utilized electronic linkage to create an ongoing dialogue among malaria researchers. We believed that the MRN can enhance exchange of information and ideas, promote multidisciplinary collaboration, sharing of reagents, and the optimum use of available resources.

The MRN is a private, multi-level global network composed primarily of scientists conducting research on various aspects of this disease. The MRN can be accessed through the World Wide Web via the home page of the Malaria Foundation (www.malaria.org). Admission to the network requires evidence of professional involvement in the field of malaria and an assigned personal password. The primary functional units of the MRN will be a series of chaired Working Groups organised around scientific disciplines. Current Working Groups include Immunology and Vaccines, Cell and Molecular Biology, Genome Studies and Genetics, Biochemistry and Pharmacology, Epidemiology and Field Studies, Vector Biology and Sporogonic Stages, Malaria Pathogenesis, Malaria Control Strategies and Mechanisms for Cooperation and Support. The chairs of the Working Groups will in turn recruit Topic Discussion leaders, who will be responsible for setting up electronic meetings organized around a specific date and time or may continue for months.

A second important function of the MRN will be the regular dissemination of information relevant to the malaria community. This will include announcements of meetings of other programs, special funding opportunities, and updates on national or international efforts in areas of malaria research and control. A third aspect of the MRN is the development of a worldwide database of reagents and resources relevant to the malaria research community.

As new wireless, satellite-based technologies make worldwide access to electronic communication more affordable and efficient, we believe that the MRN will provide many more opportunities for global collaboration and cooperation in malaria research and control.

S - 18.6

THE "OTHER" MALARIA PROBLEMS

Paul Hagan
European Commission, Brussels, Belgium

Sir Ronald Ross solved one malaria problem, but many other problems remain. The centenary of his discovery of the malaria parasite in the mosquito is an appropriate time to analyse the current situation in malaria research and control, and to plan for the fu-

ture. What lessons can we learn from the past? What changes will make a difference in the future? Whose responsibility is malaria anyway?

The INCO-DC Programme of the European Commission is a major supporter of malaria research and control activities. What is it doing to contribute to the solution of the other malaria problems?

S - 18.7

ROTARY AGAINST MALARIA

Brian Hanley
Rotary Against Malaria, 83 Black Butts Rd French Forest Sydney NSW 2086 Australia

Rotary Against Malaria (RAM) programmes are operating in the South West Pacific, in parts of Africa, and in Central land South America. Rotary International's Polio Plus programme is expected to conclude around the year 2,000. RAM aims to be strong enough to succeed Polio Plus as the next major programme of Rotary International. RAM has recognized world experts on its Scientific Advisory Board, is contributing support to vaccine and new drug development, and is seeking a 3H grant (Health, Hunger & Humanity) from the Rotary Foundation, which is one of the world's largest private charitable organizations. RAM has been endorsed by all 23 Australian Rotary Districts, as well as in New Zealand, parts of the United States, southern Africa and Switzerland, and has good relations with the World Health Organization, the Malaria Foundation and others.

S - 18.8

THE CASE FOR A CONTINUUM : THE BENCH, THE FIELD THE POLITICAL ARENA, AND CONTROL

Daniel G Colley
Malaria Foundation, 3 Washington Square Village, Suite 10F, New York - 110012

Research and control is an often-used phrase that is rarely implemented in any cohesive manner. The field of malaria is not better or worse in its pursuit of the goal of integrating research and control than most other areas of global public health. The situation is simply more pressing for malaria than most others, for here failure to heed the need is certain to worsen the current, already disastrous, state of affairs. What is needed is understanding, respect, acknowledgement, coordination, and support for overall efforts applied to control, treatment, and prevention of malaria, globally. Scientists, health care professionals public health officials, administrators, and politicians must try to understand each other sufficiently to respect each other for the essential contributions they must each make to his effort. There must be mutual acknowledgment by all participants of the need for each others' contributions, and an overall recognition of the dire straits in which we find ourselves now and in the predictable future, in record to malaria. This

is an essential starting point for the crafting of new creative and effective partnerships through which to launch this critical offensive. The continuum of contributory disciplines that is needed will require difficult-to-implement, but essential, coordination and there must be something to coordinate. Can this be done within the existing format and boundaries used previously for such efforts ? Can the multitude of necessary academic, industrial, philanthropic, governmental, and non-governmental partners agree on how to tackle this challenge, and then do it? The gauntlet thrown before us may require the development and use of new contexts and approaches, or it just may need good old-fashioned leadership, consensus building, and a joint sense of mission. It will undoubtedly require a higher level of unity, cooperation, and mutual respect among all those involved, than now exists. We have to prepare ourselves to face the new changes in the epidemiology of malaria and develop systems at the national, regional and global levels to incorporate new concepts and tools into malaria control programs. This can be realized if we share the failures and successes of malaria control on a real time basis within nations, between nations in a region, and between regions in the world. The new "Spirit of 1997" — infused as it is with multilateral partnerships — that has emerged during this, the 100th anniversary year of the discovery malaria parasites in mosquitoes, provides an excellent springboard from which to launch the effort needed. We most not allow this opportunity to dissipate. We must create the essential continuum through constant bridge building and repair — beginning now.

S - 19.1

USE OF SYNTHETIC PEPTIDES IN MALARIA VACCINE DEVELOPMENT

V S Chauhan
International Centre for Genetic Engineering & Biotechnology, Aruna Asaf Ali Marg, New Delhi-110 067, India

Resistance of the human malaria parasite to common antimalarials, and to its mosquito vector to insecticides has greatly worsened the malaria situation. A malaria vaccine will be of immense value in combating malaria. Malaria vaccine research offers a great scientific challenge and has traditionally been in the forefront in the development of new technologies applicable to human health. Several malaria antigens are being investigated as vaccine targets but it seems that immunodominant domains of these proteins do not always contribute to protective immunity. Peptide based vaccine constructs offer a reasonable choice even though there are inherent problems in their use.

We have been interested in developing linear peptides containing multiple B and T cell epitopes as vaccine candidates, and as models to study underlying principles correlating their structure with immune functions. We have found that in a multiple epitope peptide based on two blood stage antigens, the orientation of B and T cell epitopes plays a crucial role in determining the immune responses. In another study, a sixty residue polypeptide based on the CSP structure, was found to be highly immunogenic and protective against a heterologous challenge with malaria infection. A mere addition of five residues at the C-terminal to include an extra T cell epitope completely shifted the focus of immune responses. Circular dichroism study revealed major differences in the overall structure of the two peptide antigens. It is clear that rules for the design of peptide based constructs remains unclear as yet.

S - 19.2

MEROZOITE SURFACE PROTEIN 1 : STRUCTURE PROCESSING AND IMPORTANCE AS A TARGET TO PREVENT RED BLOOD CELL INVASION

Anthony Holder, Mike Blackman, Sue Fleck, Jose Guevara, Irene Ling, Sola Ogun, Terry Scott-Finnigan, Lilian Spencer and William Stafford
National Institute for Medical Research, Ridgeway, Mill Hill, London-NW7 1AA

Plasmodium falciparum merozoite surface protein 1 (MSP -1) is synthesized as a precursor and then processed to a series of fragments in a multimeric complex containing two other unrelated polypeptides of 36 and 22 kDa on the surface of the merozoite. At or just before erythrocyte invasion the complex is shed from the surface as the result of a second proteolytic cleavage within the C-terminal 42 kDa fragment that has a GPI anchor to the plasma membrane. Some monoclonal antibodies (mAbs) that bind to the C-terminal 19 kDa product of this secondary processing of MSP-1 inhibit invasion and appear to do so by interfering with the proteolytic cleavage. Immunization of mice with

a recombinant protein corresponding to the C-terminal 19 kDa region of *Plasmodium yoelii* MSP-1 or passive immunization with mAbs specific for this protein, result in protection against blood stage challenge infection with this rodent parasite. Antibodies binding to some other parts of *P. falciparum* MSP-1 blocking the binding of the inhibitory mAbs to the C-terminal region, suggesting that the balance between inhibitory and block antibodies may be critical for the effectiveness of an immune response to MSP-1. Although, the function of MSP-1 is unknown, it provides a good target for intervention to interfere with erythrocyte invasion by the parasite.

S - 19.3

AMA-1 OF P. FALCIPARUM AND P. VIVAX : RECOMBINANT EXPRESSION FOR SUB-UNIT VACCINE DEVELOPMENT AND HETROLOGOUS EXPRESSION THROUGH TRANSFECTION OF MALARIA PARASITES

Alan Thomas[1], Annemarie van der well, Jack Pronk2, Martin Dubbeld[1], Andrew Waters[3], Chris Janse[3] jan Langermans[1] and Clemens Kocken[1]
1. Dept. Parasitology, Biomedical Primate Research Centre, Lange Kleiweg 157, 2288GJ Rijswijk, The Netherlands
2. Kluyers Laboratory of Biotechnology, Industrial Biotechnology Section, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands 34 Department of Parasitology, University of Leiden, Wassenaarseweg 62, 2300 RC Leiden, The Netherlands.

The Apical membrane antigen-1 (AMA-1) family of molecules are merozoite antigens of undetermined function that appear to be intimately involved in red cells invasion. Two forms of AMA-1 have been described, an 83 kDa form present in P. falciparum and the closely related chimpanzee malaria P. reichenowi, and a 66kDa from that is present in all other malaria species analysed to date. The difference in molecular weight is accounted for primarily by an insertion/deletion in the N-terminal regions of the molecule. In simian and mouse models AMA-1 vaccination has induced protective immune responses, althoug there is evidence from the mouse model that regionally localised point mutations may have an impact on vaccine outcome. From vaccine studies and in vitro assays of parasite multiplication in the presence of antibodes to AMA-1 it appears that the conformation of AMA-1 is critical to protective effects. Di-sulphide bonds have recently been assigned, and a protocol to re-fold prokarytically expressed P. falciparum AMA-1 is currently being used to generate material for clinical trials. Another approach is to use eukaryotic expression system which do not require refolding. We have used the yeast Pichia pastoris for high level expression of secretion products of the P. vivax 66 kDa AMA-1 ectodomain (PV66/AMA-143-487) which do not require refolding. Purified PV66Dglyc43-487 is highly immunogenic in rhesus monkeys in combination with the adjuvant SBAS2.

To develop systems to better analyses, for example, structural unctional relationships we have used AMA-1 as a lead molecule. We have transfected the rodent malaria P.

berghei with the AMA-1 of P. falciparum (PF83/AMA-1). Stable expression of correctly folded PF83/AMA-1 has been obtained with a stage specific and subcellular distribution that strongly depends on protmoter contol. Using the AMA-1 promoter of P. berghei. to the molecule similar to that seen in P. falciparum, indicating that sequences influencing localisation are conserved between species. Processing of PF83/AMA-1 also occurs in P. berghei.. Mice infected with the transacted P. berghei produce high levels of PF83/AMA-1 specific antibodies. Similar results have been obtained by transfection of the macaque monkey malaria parasite P. knowesi with heterologous AMA-1 species. This shows that heterologous expression of critical malaria molecules between phylogenetic distant parasites is feasible, and holds promise for a deeper understanding of structural and functional relationship for critical vaccine and drug targets.

We gratefully acknowledge the provision of adjuvant by Smithkline Beecham Biologicals s.a., Rue de L' Institut 89, B-1330 Rixensart, Belgium

S- 19.4

HUMAN PHASE I VACCINE TRIALS OF 3 RECOMBINANT ASEXUAL STAGE MALARIA ANTIGENS.

Allan Saul, GL Lawrence, A Smillie, C Rzepczyk, K Anderson, R Anders, G Brown, D Pye, D Irving, R Reber, and D Sturchler.
Qld Institute of Medical Research and Royal Brisbane Hospital, Brisbane, Qld. Australia.
Walter & Eliza Hall Institute of Medical Research, Melbourne, Vic, Australia.
CSL Ltd., Melbourne Vic, Australia.
Biotech Australia, Sydney, NSW, Australia.
F Hoffmann-La Roche Ltd, Basel, Switzerland.

Two phase I vaccine trials have been conducted to test the immunogenicity and safety of a vaccine comprising three recombinant malaria antigens from the asexual stage of *P. falciparum* in Montanide ISA 720 adjuvant. The three antigens are 190L, a fragment of MSP-1; MSA-2 and portion of RESA MSP-1 and MSA-2 are merozoite surface antigens. RESA is present in merozoites and is associated with the red cell cytoskeleton following invasion. The Montanide ISA 720 adjuvant is produced by SEPPIC and is a metabolizable oil with a mannide mono - oleate emulsifier that has given promising activity in animal trials and developed for human use. These trials investigated the dose response of each antigen for eliciting both antibody and T cell responses and the efficacy of a mixture of the antigens compared with the antigens injected separately. The responses of the MSA2 component in Montanide ISA720 were also compared with the responses obtained in an earlier trial that used alum as the adjuvant.

All three antigens elicited both antibody and T cell responses. Particularly strong T cell responses were observed with 190L and RESA. Stimulation indices exceeded 100 for peripheral blood leucocytes in some individuals. By contrast, the antibody responses,

although significant, were substantially lower than had been observed in trials with small animals. The antibody responses observed with MSA2 were not significantly different in the volunteers in this trial with Montanide ISA720 adjuvant than previously observed with alum. No antigenic competition was observed; volunteers receiving a mixture of antigens had similar responses to those receiving the three antigens at separate sites.

S - 19.5

MALARIA DNA VACCINE DEVELOPMENT

Stephen L Hoffman,[1] Denise L. Doolan,[2] Martha Sedegah,[3] Ruobing Wang,[4] *Sanjai Kumar,*[1] Libia F Scheller,[1] Anita Kumar,[4] Walter R Weiss,[1]Thong P Le,[1] Dennis M Klinman,[5] Peter Hobart,[6] Jon A Norman,[6] and Richard C Hedstrom[1].
1. Malaria Program, Naval Medical Research Institute, Bethesda, MD 20889-5607.
2. Pan American Health Organization, Washington D C 2003.
3. University of Maryland, Baltimore, MD 21201-1559.
4. Henry M Jackson Foundation, Rockville, MD 20892.
5. Center for Biologics Research and Evaluation, Food and Drug Administration, Bethesda, MD 20892.
6. Vical Incorporated, San Diego, CA 92121.

In July, 1997 the first DNA vaccine designed to protect against malaria was administered to human volunteers. This single gene DNA vaccine encoding the *Plasmodium falciparum* circumsporozoite protein (PfCSP) is being studied for safety and immunogenicity. If these criteria are met , a multi-gene DNA vaccine designed to induce protective $CD8^+$-T cell responses against *P. falciparum* infected hepatocytes will be subsequently assessed for safety, immunogenicity and sporozoites. In parallel, we are also developing multi-gene *P.falciparum* erythrocytic stage DNA vaccines that are being assessed in *Aotus* monkeys. Our perspectives on malaria vaccine development in general and on a multi-gene DNA vaccine in particular will be discussed. The rationale and experimental foundation for the anticipated *P. falciparum* DNA vaccine trials will also be reviewed.

S - 19.6

GAMETOCYTES

Richard Carter
Institute of cell, Animal and Population Biology University of Edinburgh, Ashworth Laboratories, King's Building, Wed Mains Road, Edinburgh, EH933J t, Scotland, U.K.

In a period of 17 years between 1880 to 1897, the discoveries of malaria parasites, their mode of transmission through mosquitoes and the sexual nature of their life cycle, laid the foundations of our understanding of the biology of malaria. Each of these discoveries crucially involved the gametocyte stage of the parasites. Not only in the early

history of malaria parasites but also in their biology and evolutionary origin the sexual stages of malaria parasites are both central the primordial. Understanding gametocytes is, therefore, at the heart of understanding the nature of the malaria parasites. In this presentation I will give a historical review of the discoveries concerning, or based on, the role of gametocytes int eh biology of malaria from those of Laveran, Ross and MacCallum through to the beginnings, one hundred years later, of the molecular exploration of gametocyte and gamete cell biology and of the controls of their differentiation and transformation processes. Developments in molecular biology and in particular the advent of practical means for transfecting malaria parasites, promise increasingly rapid progress in the breadth and depth of our understanding of their sexual stages, the gametocytes.

S - 19.7

S Jepsen
(Abstract not received)

S - 19.8

RATIONAL DESIGN AND DEVELOPMENT OF ADJUVANT-ACTIVE NONIONIC BLOCK COPOLYMERS

Mark J Newman and Charles W Todd
Vaxcel, Inc., 154 Technology Parkway, Norcross, GA 30092 USA

Nonionic block copolymers are surfactants synthesized using oxypropylene and oxyethylene and are synthesized so that cores of polyoxypropylene (POP) are flanked by smaller polyoxyethylene (POE) end blocks. These copolymers are linear with the polymer blocks organized as POE-POP-POE. The adjuvant activity of nonionic block copolymers is significantly influenced by the size of the POP core block, as the size of the POP core is increased so is the adjuvant activity with the peak activity is achieved using copolymers with POP cores that are 12-15 kDa. However, adjuvant potency and the type of immune responses produced are also influenced by the POE content. Copolymers with 10% POE significantly augment Type 2 helper T-lymphocyte responses whereas copolymers with 5% POE augment both Type 1 and Type 2 helper T-lymphocyte responses. Copolymers synthesized with less than 10% POE will spontaneously assemble into 500nm - 3µm micelles or microparticles in aqueous solutions at physiological pH and when formulated with protein. These nonionic block copolymers can therefore be used in vaccine formulations without other surfatants or oils which significantly reduces the potential for systemic and localized toxic reactions.

S - 20.1

SEQUESTRATION OF P. FLACIPARUM INFECTED ERYTHROCYTES TO CHONDROITIN SULFPHATE A: DESQUESTRATION BY SOLUBLE CSA IN VIVO AND CLONING OF THE PARASITE COUNTER-RECEPTOR

Scherf A[1], Pouvelle, B[2], Hernandex-Rivas, R[3] , Boffa, MC,[4] and Gysin, J[2].
1. Institut Pasteur, Paris; 2. Institut Pasteur, Lyon; France; 3. CINVESTAV-IPN Mexico., 4. INSERM Unit 353, Hospital Saint Louis, Paris, France.

We described recently the adhesion of infected red blood-cells (IRBC) to the glycosaminoglycan chondroitin sulphate a (CSA) of endothelial cells isolated from Saimiri brain and human lung capillaries Lately, it wa shown that CSA also mediates cytoadherence to human placentas and appears t be involved in complications linked to malaria during the first pregnancy, We present evidence that thrombomodulin, - an endothelial cell surface proteoglycan rich in CSA is an adherence receptor for IRBC, Soluble CSA was cited into P. falciparum infected Saimiri monkeys which resumed in a release of mature parasites into the circulation The desequestered parasites show specific binding to CSA, - 20

The Plasmodium falciparum erythrocyte surface protein 1 (EMP1) is the principal parasite derived-protein mediating adherence of IRBC to endothelial cells, EMP1 is encoded by a multigene family called var. In order to identify the parasite-encoded ligand for CSA, we selected parasites from the he FCR3 clone on a Saimiri endothelial cell line expressing exclusively CSA as cytoadherence receptor. After four rounds of panning, IRBC adhesion to CSA could be efficiently inhibited by soluble CSA and by treatment of the endothelial cells with chondroitinase, FCR3-CSA selected parasites adhered specifically to CSA and not to endothelial cells expressing CD36 or ICAM-1, An abundant transcript was detected in RNA preparation of FCR3-CSA parasites which was absent in the FCR3 strain before panning or FCR3 parasites panned to CD36 or ICAM-1. Molecular cloning and DNA sequence analysis of this transcript revealed significant homology to the var gene family. Antisera were rased against recombinant fusion proteins and synthetic peptides corresponding to the gene product. We will report the capacity of these antibodies to inhibit parasite adhesion to CSA.

S - 20.2

CONSTRUCTION AND CHARACTERISATION OF A PLASMODIUM VIVAX GENOMIC LIBRARY IN YEAST ARTIFICIAL CHROMOSOMES.

Hernando A del Portillo, Anamaria A Camargol, Carmen Fernadez-Becerral, Daniela M F Coural, Katja Fischer2, Michael Lanzer2 and
1. Departmento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Av. Lineu Prestes 1374 Sao Paulo, SP 05508-900 Brazil.
2. Zentrum fur Infektionsforschung, Univesitat Wurzburg, Rontgenring 11, D-97070 Wurzburg, Germany.

Plasmodium vivax is the most widely distributed human malarial parasite causing an estimated 35 million cases annually. In some parts of the world, including Brazil where it reaches almost 70% of all the yearly malaria cases, this is the most prevalent species. Here we describe the construction of a representative YAC library for the human malarial parasite P. vivax. As P. vivax can not be maintained continuously under laboratory conditions, the P. vivax DNA necessary for the library, construction was isolated from a single human patient presenting himself to a local hospital in the Brazilian amazon with vivax malaria. Thus, this YAC library is the first of its kind to be generated from patient-derived material. The YAC library consists of 560 clones with an average insert size of 180 kb. Of nine published P. vivax genes, eight were found to be present in the library. In addition, twelve P. vivax telomeric YAC clones were identified and partial characterisation of them will be presented.

S - 20.3

MAPPING OF SILENT AND TRANSCRIBED *VAR* GENES ALONG *PLASMODIUM FALCIPARUM* GENOME

Michael Lanzer
Zentrum fur Infektionsforschung der Universitat Wurzburg, Rontgenring 11, D-97070 Wurzburg, Germany.

P. falciparum var genes encode a diverse family of proteins, located on the surface of infected erythrocytes, which are implicated in the pathology of human malaria through antigenic variation and adhesion of infected erythrocytes to the microvasculature. We have constructed a complete representative telomere to telomere yeast artificial chromosome (YAC) contig map of the *P. falciparum* chromosome 8 for studies on the chromosomal organization, distribution and expression of *var* genes. Three *var* gene loci were identified on chromosome 8; two of which map close to the telomeres at either end of chromosome. Analysis of the previously described chromosome 2 contig map and random *P. falciparum* telomeric YAC clones revealed that most, if not all, 14 *P. falciparum* chromosomes contain *var* genes in a subtelomeric location. Mapping the chromosomal location of *var* genes expressed in a long term culture of the *P. falciparum* strain Dd2 has revealed that 4 out of the 5 different expressed *var* genes from a chromosomal domain known for frequent rearrangements has important implications for the mechanism of *var* gene switching and the generation of novel antigenic and adhesive phenotypes.

S - 20.4

SEQUENCING THE *P. FALCIPARUM* GENOME : STRATEGIES, PITFALLS AND SUCCESSES.

Daniel Lawson, and Bart Barrell.
The Sanger Centre, Wellcome genome Campus, Hinxton CB10 ISA.

Plasmodium falciparum has an estimated genome size of 30 Mb. We have been focusing on chromosome 3 as a pilot project for sequence analysis because of its small size, 1.2 Mb, and good resolution on pulsed field gels. Malaria DNA has a A+T content of 81% which appears to render large fragments unstable in bacterial cloning systems such as cosmids. Hence, no bacterial clone map exists for the genome. However, the DNA can be cloned in yeast, and the Wellcome Trust Genome Mapping Project is close to completing a YAC based physical map.

Initially, we had no experience of direct YAC sequencing and no knowledge about the frequency of YAC rearrangements, so a whole chromosomal shotgun strategy was adopted. Gel Purified material is shotgun cloned into the pUC vector system and clones sequenced randomly obtaining both forward and reverse reads. 32 000 reads have been generated from a number of libraries with an estimated chromosomal purity of 87% based on hybridisation experiments. Assemblies of the shotgun yields a dataset of contigs totalling 1.27 Mb. This total excludes singleton reads which are expected to be contamination from other chromosomes. Light shotguns of YAC clone covering the chromosome has been used to order and group contigs. Contiguation of shotgun sequences is achieved by directed sequencing reactions (oligo walking), and PCR followed by small insert pUC libraries for gap filling.

We experienced problems with cloning bias in the first libraries; telomeric and subtelomeirc repeat sequences were cloned preferentially because of their less extreme A+T content (70%) which appears to make them more stable and less liable to damage during DNA isolation. The elimination of the heating step in agarase digestion of gel plugs has proved to reduce the risk of DNA damage and subsequent libraries did not show the same degree of bias.

Preliminary analysis of the contigs has confirmed the chromosomal location of a number of characterised gene, namely CSP, CTRP, RNA polymerase largest subunit and the 204 kDa antibody recognised protein. Further all but one of the mapped STS sequences has been located and new STSs are being generated from this dataset. We estimate that the chromosome encodes 200 - 300 genes based on analysis of long open reading frames and BlastX similarities to the public databases.

The shotgun data is available via the Sanger Centre Web site (http: // www.sanger.ac.uk/ Projects/P_falciparum) where similarity searches can be run or pre-calculated results browsed. Partially edited sequence contigs are submitted to the public nucleotide

databases as part of the High Throughput Genome Sequence (HTGS) section. Preliminary analysis of config sequences will be put on the web site as appropriate.

S - 20.5

CURRENT STATUS OF THE *P. FALCIPARUM* SEQUENCE TAG PROJECT

G Roman Reddy, John B Dame, Debopam Chakrabarti Susan M. Tanhauser, Sheldon M Schuster, and Nathan Goodman
Department of Pathobiology, University of Florida, Gainesville, FL USA 32611-0880

Plasmodium falciparum is the most dangerous and rapidly proliferating of the four species of the malaria parasite parasitic in man. It infects 300-500 million people and kills nearly 2 million annually worldwide. Identification, sequencing and mapping of the structural genes from this parasite provide data from which is launch applied research programs for antimalarial drug and vaccine development. A novel, genes-first approach to genome sequencing is utilized which efficiently generates gene sequence tags from genomic DNA as well as cDNA from the asexual blood stages. Under modified reaction conditions, mung bean nuclease (EC 3.1.30.1) cleaves genomic DNA of many organisms, including *P. falciparum,* before and after genes and within some introns. This distinctly new approach to genome sequence characterization has been carefully evaluated by examining a large number of fragments. Results indicate that this method has several advantages over using the expressed sequence tag approach (cDNA) alone. The status of this project was recently reviewed and is available in a regularly updated form on the Internet at http://parasite.arf. ufl.edu. To date, > 1,700 gene sequence tags have been generated from > 1,300 clones comprising nearly 700 kb of new sequence. Nearly 20% have significant similarity to sequences in the protein and translated nucleic acid database. The genes identified include proteins expressed in erythrocytic, exoerythrocytic and gametocytic stages of the parasite. The spacer DNA separating genes is rarely cloned. Nearly, ninety percent of the clones with no homology have long open reading frames suggesting that majority of them code for proteins. In addition, > 1,100 expressed sequence tags have been prepared. These expressed sequence tags provide an additional 331 kb of sequence data and a similar percentage are identified by sequence homology. Together these data sets provide over 1 megabase of sequence and tags for 20% of the genes of the parasite. These sequence tags are being used to initiate projects to develop new therapeutics, vaccines and strategies to control human malaria.

S - 21.1

PROBLEM OF SYMPTOMATIC ANDz ASYMPTOMATIC GIARDIASIS

R C Mahajan, N K Ganguly, Ajaib Singh and Anuradha Chakarvarti
Depts.. of Parasitology & Experimental Medicine & Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh - 160 012, India

Giardiasis which is essentially a disease of children and causes diarrhoea in children below 5 years, presents itself in 75% of children as asymptomatic infection. There have been many attempts to differentiate the strains of *Giardia lamblia* into two groups, symptomatic and asymptomatic, through isoenzyme patterns or through several genes including giardin.

However, the results have not been consistent. We have recently cloned a 0.8 Kb fragment of *Giardia lamblia* from a strain axenised from a case of symptomatic giardiasis. This was identified through immunoscreening using antibodies from symptomatic and asymptomatic patients of giardiasis. A primer was obtained after sequencing the gene which could differentiate symptomatic from asymptomatic strains. A dot blot hybridization has successfully been developed as a test to differentiate strains isolated from symptomatic and asymptomatic patients.

S - 21.2

HETEROGENEITY OF DNA CONTENT AND EXPRESSION OF CELL CYCLE GENES IN AXENICALLY GROWING *ENTAMOEBA HISTOLYTICA* HM1 : IMSS CLONE A

Anuradha Lohia[1], Samudra S Gangopadhyay[1], Sib Sankar Ray[1], Kavin Kennady[2], Gopal Pande[2]
1. Department of Biochemistry, Bose Institute, Calcutta - 700 054, India
2. Centre for Cellular and Molecular Biology, Hyderabad - 500 007, India

The cell division cycle of *Entamoeba histolytica* was studied using multi parametric flow cytometry in asynchronous and partially synchronised cells. Dynamic changes in the DNA synthesis and DNA content of axenically growing trophozoites were observed by using 5-bromo-2'-deoxyuridine (BrdU) uptake and DNA specific fluorochromes. It was observed that DNA synthesis in these cells continues beyond the fluorochromes. It was observed that DNA synthesis in these cells continues beyond the typical S-phase stop point when DNA duplication is complete. Asynchronously growing *E.histolytica* cells could be synchronised by serum starvation followed by serum re-addition. BrdU incorporation in synchronised cells showed that cell synchrony is maintained for at least one generation time, in which the G1 phase lasts for 2-3h and the S-phase lasts for 5-6h. Analysis of our results revealed that *E.histolytica* trophozoites, growing in axenic medium, are made up of a heterogenous population of euploid and polyploid cells. The number of polyploid cells increases with age of the cells in culture. Expression of

putative cell cycle and signal transduction markers was studied using specific antibodies and changes in their expression levels have been correlated with changes in the DNA content. Based upon our results we could identify G1, S and G2 phases of the cell cycle of *E.histolytica* and also predict the mechanism underlying the generation of polyploidy in these cells, which may have significant effects on its biology and pathogenesis.

S - 21.3

P Das
(Abstract not received)

S - 21.4

S Bhattacharya
(Abstract not received)

S - 21.5

A Chakaravarti
(Abstract not received)

S - 21.6

H Kamra
(Abstract not received)

S - 22.1

D Krogstad
(Abstracts not received)

S - 22.2

MITOCHONDRIAL FUNCTIONS AS TARGETS FOR ANTIMALARIAL DRUGS

Akhil B Vaidya, Indresh K Srivastava, Joanne Morrisey
MCP/Hahnemann School of Medicine, Allegheny University of e Health Sciences, Philadelphia, PA 19129, USA.

Malarial parasites contain unusual mitochondria. They lack citric acid cycle enzymes and are generally without elaborate cristae. Yet, proper functioning of mitochondria is essential for parasite survival. In addition to supporting pyrimidine biosynthesis, malarial mitochondria are likely to be critical for a number of metabolic processes. Through sequence analyses of the 6-kb mitochondrial DNA of malarial parasites, we have uncovered unique structural features of the cytochrome b, and proposed these to be the targets for antimalarial drugs such as hydroxynaphthoquinolines. We have also developed assays for mochondrial physiology and functions in live intact parasites. Using these assays, we found that atovaqune collapsed mitochondrial membrane potential ($\Delta\psi_m$), and inhibited mitochondrial electron transport within minutes. We have derived atovaquone-resistant *P. yoelii* through suboptimal treatment obtaining several independent isolates. The resistant parasites were also refractory to atovaquone-mediated collapse of $\Delta\psi_m$ and electron transport inhibiton. Sequencing the mitochondrial DNA revealed two clustered amino acid changes within the loop connecting transmembrane helices 5 and 6 of cytochrome b. These results provide a molecular model for atovaqu action and resistance in malarial parasites. We have also examined the effects of atovaquone/proguanil combination on mitochondrial physiology. Our results suggest that this combination of drugs works through mechanisms indendent of the electron transport and dihydrofolate reductase inhibition. If true, this may lead to identification a hitherto unknown target for malaria chemotherapy.

S - 22.3

RESEARCH ON NEW ANTIMALARIAL DRUGS IN THE BANGKOK HOSPITAL FOR TROPICAL DISEASES

Sornchai Looareesuwan
Department of Clinical Tropical Medicine and Hospital for Tropical Disease, Faculty of Tropical Medicine, Maltidol University, Bangkok, Thailand.

With the emergence of multidrug resistant falciparum malaria in Thailand. New drug and drugs in combination the urgently needed. New antimalrial drugs have been inves-

tigated at the Hospital for Tropical Diseases in the recent years. Atovaquone, A hydroxynaphthoquinone, was evaluated and found that atovaquone alone proved safe and effective. All patients treated had clinical cure, however, one thrid of patients had late recrudesence (RI). When it was combined with proguanil, the cure rate increased to 100%. Artemisinine derivatives such as artesunate, artemether, arteether, dihydroartemisin in are also tested at the Bangkok Hospital for Tropical Diseases. Artesunate and artemether alone with a total dose of 600 to 750 mg produced cure rates of 80 to 95%. Artesunate suppositories have been proved successfully for the treatment of sever malaria. The artemisinin derivatives when used in combinations with mefloquine cure rates improved to 95-10%. Dihydroartemisinin alone with a tototal dose of 480 mg given over 5 days gave a cure rate of 90%. Arteether, a WHO/TDR supported drug, are being evaluated in phase III clinical trial for severe falciparum malaria in the hospital. Other combinations (artemisinin derivatives combined with doxycycline, mefloquine combined with tetracine or doxycycline) have also been evaluated with the improvement in cure rates. At present, studies with the comon of artemisinin derivatives plus mefloquine in various doses and duration of treatment, are being investigated. Until proven otherwise, the drug combinations are still recommended for all adult patients suffering from acute uncomplicated falciparum malaria contracted in multiding resistant areas. Other antimalarial drugs such as pyronaridine and new 8 aminoquinotone (WR238605) are being evaluated.

S - 22.4

EFFECTORS OF THE PLASMODIAL PHOSPHOLIPID METABOLISM : DEVELOPMENT OF A PHARMACOLOGICAL MODEL

Henri J Vial
Molecular Dynamics of Membrane Interactions, CNRS UMR 5539, University of Montpellier II, Case 107, 34095 Montpellier Cedex 5, France.

Due to the increasing *Plasmodium* resistance to the various conventional antimalarial agents, novel pharmacological models are required. Parasite phospholipid metabolism is an original target and novel antimalarial drugs have been designed, based on interference with this essential metabolism developed by *Plasmodium* during its intraerythrocytic cycle. The most promising drug interference is choline transporter blockage, which provides *Plasmodium* with a supply of precursors for the synthesis of phosphatidylcholine, the major phospholipid of infected erythrocytes.

In vitro antimalarial activity against resistant *P. falciparum* strains and isolates, *in vitro* activity against *P.vivax*, absence of *in vitro* resistance induction under long term drug pressure, *in vivo* activity against various murine species and *P. falciparum* - infected *Aotus* monkeys even at very high parasitemia and without recrudescence, absence of genotoxicity, are the most prominent characteristics of these new molecules. Very recent data concerning *P. falciparum*-infected *aotus* monkeys will be detailed. The antimalarial activity of the compounds is satisfactory All these considerations mean

that the approach is now quite realistic.

We are currently studying PL metabolism metabolic regulation. These studies concern potential mechanisms of parasite resistance acquisition when choline supply is blocked by pharmacological interference, along with the discovery of metabolic pathways that could be absent from mammalian hosts.

S - 22.5

ACCELERATED DRUG RESISTANCE IN RECENT ISOLATS OF *PLASMODIUM FALCIPARUM*

Pradipsinh K Rathod, Pei-Chieh Lee, and Dennis Kyle*
The Catholic University of America, Department of Biology Washington, D.V. and * Walter Reed Army Institute of Research, Division of Experimental Therapeutics, Washington, D.C.

The widespread emergence of drug resistance in *Plasmodium falciparum* raise important questions related to the initiation of drug resistance. It is now possible to estimate variations in frequencies of drug resistance in *P. flaciparum* using the antimalrial agents 5-fluoroorotate or atovaquone (Gassis, S. and Rathod, P.K. 1996. Antimicrob. Agents hemother. 40 : 914-919). Some laboratory clones of *P. falciparum* having preexisting resistance to a large number of additional antimalarial agents acquired resistance to new compounds much more easily than parasites that were sensitive to common antimalarial agents (Rathod, P.K., McErlean, T., and Lee, P.-C. Proc. Natl. Acad, Sci. (USA), in press). Present studies demonstrate that accelerated Resistance to multiple Drugs (ARMD) phenotypes not unique to old laboratory clones : they are prevalent recently isolated, highly drug-resistant parasites from Thailand.

Supported by NIH grants RO1 AI-26912 and
a Burrough Wellcome Fund New Initiatives in Malaria Research Award.

S - 22.6

RECENT DEVELOPMENTS ON NEW 8-AMINOQUINOLINE ANTIMALARIALS FOR MALARIA PROPHYLAXIS AND RADICAL CURE

S K Puri and G P Dutta
Division of Microbiology, Central Drug Research Institute, Lucknow

The effective management of drug resistant strains of *P. falciparum* malaria, which is now extending to *P. vivax* also, is a steadily increasing clinical problem underlying importance of continued search for alternative remedies and strategies. 8-Aminoquinoline drugs represent the only class of compounds producing parasiticidal effect on asexual blood and tissue dwelling forms as well as the sexual stages of the parasite. Experimental studies at CDRI during the last 15 years were instrumental in pre-clinical develop-

ment of two potential 8-aminoquinoline antimalarials CDRI 80/53 and WR 238605 for treatment of relapses. CDRI 80/53, an enamine analog of primaquine, has completed Phase II clinical trial in *P. vivax* cases while identical trials with WR 238605, a 4-methyl 5-phenoxy primaquine derivative synthesized at WRAIR, are in progress under WHO-WRAIR sponsorship. Additional spectrum of activity of these two potential compounds has been explored in the simian models. Efficacy tests with WR 238605 revealed marked blood schizontocidal activity against *P.cynomolgi* and *P.fragile* and an additive /synergistic response in combination with halofantrine. This combination would be useful alternative regimen for chemotherapy of chloroquine resistant *P. vivax* cases. Both CDRI 80/53 and WR 238605 have shown promising gametocytocidal activity for interruption of transmission of drug-resistant strains. Compound 80/53 has also shown causal prophylactic activity against *P. cynomolgi* sporozoite challenge. In view of relative safety of CDRI 80/53 over primaquine, its prophylactic usage in endemic areas is warranted. CDRI 80/53 may provide a safe and effective prophylaxis against drug-resistant *P. vivax* and *P. falciparum.*

S - 22.7

CYSTEINE PROTEASE INHIBITORS FOR THE TREATMENT OF MALARIA

Philip J Rosenthal, Jed E Olson, Andrey Semenov and Jose N Dome EDnguez
Dept. of Medicine, University of California, San Francisco, CA, USA
Facultad de armacia. Universidad Central de Venezuela, Caracas, Venezuela

Erythrocytic malaria parasites degrade hemoglobin to provide free amino acids for protein synthesis. We have shown that cysteine protease activity is required for hemoglobin degradation and we have characterized the trophozoite cysteine protease falcipain as a *P. falciparum* haemoglobinase. We are currently evaluating the antimalarial effects of falcipain inhibitors. With peptide-based compounds, including fluoromethyl ketones and vinyl sulfones, the *in vitro* inhibition of falcipain correlated with inhibition of both globin hydrolysis and growth of cultured parasites. The most potent inhibitors blocked globin hydrolysis and parasite development at low nanomolar concentrations. In studies utilizing a P. vinck) IwwwX"H7 by a molecular modeling approach. Among these compounds, a series of chalcones inhibited parasite development at nonmoral concentrations and series of phenothiazines inhibited falcipain, blocked parasite hemoglobin degradation and inhibited parasite development at micromolar concentrations. Our results suggest that the rational design of inhibitors of falcipain may be promising strategy for the development of new antimalarial chemotherapeutic agents.

S - 22.8

CHLOROQUINE TRANSPORT AND PROTON EXCHANGE ARE LINKED IN *PLASMODIUM FALCIPARUM*

Michael Lanzer
Zentrum fur Infektionsforschung der Universitat Wurzburg, Rontgenring 11, D-97070 Wurzburg, Germany

Chloroquine was the first choice antimalarial drug for more than three decades until the emergence and spread of chloroquine resistant *Plasmodium falciparum* strains rendered its application ineffective in many parts of the world. We demonstrate that uptake of the antimalarial drug chloroquine is temperature-dependent, saturable and inhabitable in *Plasmodium falciparum*. These features are indicative of carrier-mediated transport and suggest that a *P. falciparum* - encoded protein facilitates chloroquine import. Although both chloroquine resistant and susceptible parasite isolates exhibit facilitated chloroquine uptake, the kinetics differ. Chloroquine resistant parasite isolates consistently have an import mechanism with a lower transport activity and a reduced affinity for chloroquine. These differences in uptake kinetics are linked with chloroquine resistance in a genetic cross. These data suggest that changes in chloroquine import kinetics constitute a minimal and necessary event in the generation of the resistant phenotype. Competitive inhibition of chloroquine uptake by amiloride derivatives further suggests that chloroquine import is mediated by a plasmodial Na^+ /H^+ exchanger.

S - 23.1

CLINICAL ASPECTS OF TAENIASIS AND CYSTICERCOSIS

G C Cook
Hospital for Tropical Diseases, London NWI OPE

Clinical problems associated with *Taenia* spp infection in *Homo sapiens* will be outlined with reference to the life-cycles of these cestode parasites. Although niclosamide has been extensively used in the past, praziquantel is currently the preferred chemotherapeutic agent - a single dose at 10 mg/kg being effective. Brief reference will be made to the human tapeworms of lesser or no clinical significance.

The major thrust will relate to cysticercosis-especially neurocysticercosis. Clinical aspects of this disease, together with improved methods for diagnosis, will be outlined. Prior to the 1980s, there was no satisfactory chemotherapeutic agent for this infection. There is now clear evidence that praziquantel 50 mg/kg daily for 15 days under corticosteroid cover is an effective chemotherapeutic regimen. An alternative agent is albendazole (15 mg/kg daily for 30 days). It may well be that shorter courses of both chemotherapeutic agents are also effective; evidence for this will be presented.

S - 23.2

CERTAIN ASPECTS OF PORCINE CYSTICERCOSIS

P E D Souza and Md Hafeez
Department of Parasitology, College of Veterinary Science Acharya N. G. Ranga Agricultural University, Triputi-517 502

The incidence of cysticercosis in pigs was studied in four districts of Andhra Pradesh. The data regarding the prevalence were collected from the Bacon factory, Gannavaram and three pig units of Andhra Pradesh. The prevalence of *Cysticercus cellulosae* based on 12, 960 pigs slaughtered from April, 1991 to March, 1995 at the Bacon factory, Gannavaram was 5.86 per cent. The prevalence was 6.89, 6.96, 5.81 and 3.23 per cent during four years and males were more commonly affected than females.

The CIEP detected 96.86 and 90 per cent of pigs found positive on meat inspection and 36.28 and 26.66 percent pigs negative on meat inspection with Antigen B, ES and PCS antigens and PCS AgB and ES antigens respectively.

ELISA detected 98 and 40,98 and 36 and 92 and 33.33 percent of pig positive on meat inspection and negative on meat inspection with PCS, Antigen B and ES antigens respectively. Cross reactions were observed in ELISA with PCS antigen and Antigen B in *C. tenuicollis* and hydatid cyst infected pigs. However, ES antigen did not show any cross reactivity.

S - 23.3

CELL MEDIATED IMMUNE RESPONSES IN CYSTICERCOSIS

Nancy Malla
Department of Parasitology, Postgraduate Institute of Medical Education & Research, Chandigarh-160 012, India

Experimental cysticercosis was successfully achieved in immunosuppressed and non-immunosuppressed, one month old pigs by feeding 20,000 and 500,000 *T.solium* eggs respectively. Significant increase in antibody response to crude soluble extract (CSE) antigen of *C.cellulosae* was found starting on 10th till 55 post infection day (PID) in infected and immunosuppressed animals and from 15th to 90th PID (till the time animals were followed) in infected and non-immunosuppressed animals. However, significant antibody response to purified antigenic fraction of *C. cellulosa* in immunosuppressed animals and to antigen B in non-immunosuppressed animals appeared later (25 and 30 PID respectively) than to crude soluble extract antigen. Significantly higher lymphocyte transformation to Con-A, crude soluble extract antigen and antigen B was observed on 60,75 and 90 PID in non-immunosuppressed and infected animals. T cell subset analysis indicated significant increase in T_4 and in T_4/T_8 ratio starting from T_8 cell population in infected animals was compared to controls.

Flow cytometric studies showed decrease of total T cells and an increase of total B cells in NCC patients but differences were not significant as compared to controls.

Cytokine responses in NCC patients indicated ThI type response, as significantly higher levels of antigen specific production of IL-2 and y-IFN was observed in patients as compared to controls.

S - 23.4

TAENIASIS AND CYSTICERCOSIS-ITS IMPORTANCE AS REGARDS HUMAN AND ANIMAL HEALTH AND CONTROL

R C Mahajan
Department of Parasitology, Postgraduate Institute of Medical Education & Research, Chandigarh

Taeniasis/ Cysticercosis is a major public health problem in several developing and even some developed countries & is a cause of great economic loss in animal products. The prevalence is influenced by many factors like sanitary excrete disposal, personal hygienic practices. method of cooking pork and beef and infection and quality control of slaughtered animals.

It is regarded as the second most important cause of intraspace occupying lesions following tuberculosis and most important cause of epilepsy both in adults and children.

Porcine cysticercosis is equally important as it affects large number of animals in pig raising establishments and thus causes economic losses.

Neurocysticercosis or eye involvement causes high morbidity and mortality. Diagnosis is established by CT scan MRI findings in conjunction with antibody antigen detection techniques. Cell mediated immune responses have been suggested to play part in the establishment of cysticerci in the infected host.

Reasonably good drugs are available to treat.

Pig has been found best experimental model for the study of drug effects, pathogenesis and immune responses.

Antigenic extracts from *T. solium* metacestodes has been shown to induce good level of protective immunity. Isolation of suitable and specific antigen would be necessary to develop a recombinant vaccine.

S - 24.1

PHASE 1 AND 2 CLINICAL TRIALS OF CANDIDATE MALARIA VACCINES

Myron M Levine
Center for Vaccine Development, University of Maryland, School of Medicine, Baltimore, MD 212101, USA

Phase 1 and 2 clinical trials of candidate vaccines aim to generate preliminary human data on the safety and immunogenicity of different vaccine dosages. In malaria vaccine development, the traditional role of Phase 2 trials has been expanded to include experimental challenges to measure, preliminarily, the efficacy of candidate (mainly preerythrocytic antigen) vaccines. Clinical trials have also provided invaluable "proofs-of-principle" of the feasibility of immunization strategies and immunologic data to guide vaccine development.

The pioneering efforts of David Clyde and collaborators at the University of Maryland in the early 1970s, laid the groundwork for subsequent clinical trials. Extrapolating from the studies of Ruth Nussenzweig and co-workers with *Plasmodium berghei* in mice Clyde *et al* exposed small numbers of volunteers to the bites of hundreds of x-irradiated, *Plasmodium falciparum*-infected mosquitoes. These volunteers, along with small numbers of controls, were challenged with non-irradiated mosquitoes bearing fully infective *P. falciparum* sporozoites. The demonstration that several of the irradiated mosquito-immunized subjects were protected against challenge with the homologous species of *Plasmodium* energized efforts to develop the first generation of sporozoite antigen-based vaccines. The cumulative experience gleaned from testing the first generations of sporozoite antigen-based vaccines such as $(NANP)_3$-tetanus toxoid, recombinant $R32tet_{32}$, R32NS1 formulated with monophosphoryl lipid A and squalene adjuvants and attenuated *Salmonella typhi* live vector expressing the circumsporozoite protein of *P.falciparum*, among others, showed that the candidates were well-tolerated and modestly or moderately immunogenic in eliciting serum antibodies. Several experimental challenges (that utilized mosquitoes infected by feeding on *P.falciparum* cultures) showed that these vaccines conferred circa 25-33% protection. Based on the results obtained with these early vaccine candidates, a decision was made by the Maryland/New York University collaborative group and by the U S Army/Navy research team to explore a new principle that "immunization" with irradiated sporozoites (delivered via hundreds of mosquito bites) could indeed elicit a high level of protection. Separate challenge studies performed independently by these two research groups corroborated the pioneer studies of Clyde. Using modern techniques of molecular biology and immunology, these investigators implicated a protective role played by cell-mediated immune responses directed against antigens of early liver stage parasites. A decade of Phase 1 and 2 trials of candidate malaria vaccines has shown their importance in assessing safety and immunogenicity, their utility in generating preliminary measures of efficacy (through experimental challenges) and of implicating possible immunologic correlates of protection.

S - 24.2

PHASE I/IIa SAFETY, IMMUNOGENICITY AND EFFICACY OF NYVAC-P17, A POX-VECTORED, MULTIANTIGEN, MULTISTAGE VACCINE CANDIDATE FOR *P. FALCIPARUM*

Chirstian F Ockenhouse
The Walter Reed Malaria Vaccine Evaluation Group, Walter Reed Army Institute of Research, Washington, D.C. 20307, USA

Candidate malaria vaccines have not been demonstrated previously to elicit consistently protective immune responses against challenge with *P. falciparum.* We believe that vaccine efficacy can be increased by inducing both humoral and cellular immune responses directed against antigens expressed by live virus vectors. NYVAC-Pf7, a highly attenuated vaccinia virus with seven *P. falciparum* genes inserted into its genome was tested in a Phase I/IIa safety, immunogenicity and efficacy trial in human volunteers. Each malaria gene inserted into a single NYVAC genome encoded proteins from the sporozoite, exo-erythrocytic, erythrocytic and sexual stages of the parasite's cycle. Volunteers received three immunizations of two different dosages of NYVAC-Pf7. The vaccine was safe, well-tolerated, but variably immunogenic. The magnitude of the antibody response to individual malaria antigens was generally poor and depended upon both the dose of vaccine and the vaccine immune status of the volunteers prior to study entry. In contrast, cellular (CTL, lymphoproliferative and cytokine) immune responses were detected in >90% of the volunteers. Of the 35 volunteers challenged with the bite of five *P. falciparum*-infected mosquitoes, one volunteer was completely protected and there was a significant delay in time to parasite patency in those groups of volunteers who received either the low or high dose of vaccine compared to control volunteers. There was significant boosting of antibody levels to blood stage antigens MSP-1 and AMA-1 in some volunteers previously immunized with NYVAC and infected with *P. falciparum* suggesting that vaccination strategies which prime an immune response may have an effect on repeated malaria infections.

S - 24.3

FIELD TRIALS OF MALARIA VACCINES : THE MAIN PROBLEMS ENCOUNTERED IN FORMULATING THE WHO/TDR GUIDELINES

Louis Molineaux
Peney - dessus, Satigny, Geneva 1242, Switzerland.

The WHO/TDR guidelines for field trials of malaria vaccines were produced in consultation with some 50 scientists, the author acting as (tentative) coordinator. The main problems encountered will be reviewed, including (1) assumptions concerning the epidemiology of endpoints, (2) respective roles of different study designs (individual *vs* community trials; double-blind randomized controlled efficacy trials *vs* effectiveness trials); (3) selection and measurements of primary and secondary endpoints (in particu-

lar the place of mortality trials and the measurement of malarial disease).

S- 24.4

C Rogier
(Abstract not received)

S - 24.5

P Smith
(Abstract not received)

S - 25.1

DEVELOPMENT OF NEW ANTIMALARIAL DRUGS IN THE HOSPITAL FOR TROPICAL DISEASES BANGKOK

Sornchai Looareesuwan
Department of Clinical Tropical Medicine and Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

In severe malaria, the choice of antimalrial chemotherapy depends on the clinical severity, the drug sensitivity of the parasites and the availability and preparation of the drug. Chloroquine is still the drug of choice for chloroquine-sensitive parasites occurring in some areas in Africa. Quinine and quinidine are the only widely available drugs which are effective against chloroquine-resistant strains. Two new synthetic antimalarial drugs, mefloquine and halofantrine are also effective against chloroquine resistant strains, but they have no parenteral formulation and cases of resistance to these drugs have already been reported. Quinghaosu (artemisinin : an ancient Chinese herbal medicine) and its derivatives have been used successfully in treating both uncomplicated and severe falciparum malaria. Their effectiveness in eliminating the parasites have been extensively documented, however, the recrudescent rate is rather high (10-30%). In Thailand drugs for the treatment of uncomplicated malaria are mefloquine in a dose of 25 mg/kg given in 2 divided doses 6 hours apart or quinine 10 mg/kg 8 hourly plus tetracycline 250 mg 6 hourly for 7 days in patients aged 8 years and over. In treating severe malaria, early diagnosis and early treatment are vital and the aim is to save patient's life. Prompt administration of an adequate and effective antimalrial drug is needed once the diagnosis is made. The antimalarial drugs of choice are quinine, quinidine, chloroquine (for chloroquine-sensitive strains) and artemisinin derivatives. Other symptomatic and supportive treatment include careful monitoring of fluid input and urine output, frequent observations for complications with appropriate treatment and good nursing care. In spite of these efforts, the mortality of severe malaria is still high.

S - 25.2
S Kumar
(Abstract not received)

S - 25.3
K K Bhutani
(Abstract not received)

S - 25.4

ARTEMISININ AND DERIVATIVES; FROM PLANT CULTIVATION TO PATIENT TREATMENT; 10 YEARS OF EXPERIENCE IN THE DEVELOPMENT OF THESE DRUGS

A Benakis

Laboratory of Drug Metabolism, Department of Pharmacology, University of Geneva, Geneva, Switzerland

Malaria is generally recognized to be one of the most serious parasitic diseases of the developing countries where it is a principal cause of mortality. Although it had been eradicated in some countries, there has been considerable resurgence, essentially due to resistance phenomena. The need for new chemotherapy compounds therefore become imperative. One very promising compound, developed in China, artemisinin (and derivatives), has now been found to be the answer to the need for effective treatment of uncomplicated, severe as well as cerebral malaria.

Although the chemical structure of artemesin is known, total chemical synthesis is not economically feasible and the only source to obtain the compound is the plant. The present problem is how to produce a sufficient quantity of the drug at a low enough price in order to be able to treat poor malaria patients in the many poor countries of the world. In confronting this challenge over the past ten years, we have taken the following approach :

- We have attempted to obtain the maximum yield of artemesin for a given cultivated surface. We were thus able, by careful breeding of the plant, to increase the plant yield from 0.09 and 0.1% to 1.4 and even 1.7%. We have opted for cultivation of the Artemesia Annua plant rather than rely on the collection of naturally growing plants because this allowed for optimization of harvesting period. We also established the most effective harvesting and leaf dissection procedures, both of which can greatly affect the final yield.
- Rational extraction and purification were developed in order to eliminate the waxes.
- New pharmaceutical form were developed, in particular thermostable suppositories as well for improving solubility by the utilization of cyclodextrine for microencapsulation of artemisinin and derivatives.
- Results of our numerous studies both in healthy volunteers and in patients have indicated that treatment with artesunate suppositories must be conducted by 4 administrations every 3 hours.
- The metabolic data suggest that artemesin derivatives are prodrugs and that their antimalarial action is due to their major metabolite, dihydroartemesinin (DHA). This metabolic pathway is essentially due to red cell reductases.
It has been observed that the injectable from of artemesinin derivatives are not effective in some malaria patients, probably because these patients may have a genetic lack of erythrocytic reductases.
- We observed by electron microscopy that five hours after administration of the artesunate in a patient, trophozoids were destroyed and DHA was present in large amount in the plasma.

Our experience in cooperation in numerous Asian, African,and South American countries has convinced us that in view of the nature of the problem these countries must be

04709

involved in the cultivation of the plant and production of the Artemisinin Compound. We are entirely willing to share our technology with them.

S - 25.5

SYNTHETIC TRICYCLIC TRIOXANE ANTIMALARIALS: SIMPLE, POTENT ARTEMISININ ANALOGUES

Charles W Jefford
Department of Organic Chemistry, University of Geneva, CH-1211 Geneva, Switzerland.

The discovery in 1979 that two sesquiterpenes derived from Chinese medicinal plants, the peroxide yingzhaosy A (1), and the trixane artemisinin (2), are active against chloroquine resistant strains of *Plasmodium falciparum*, has opened a new era in the chemotherapy of malaria. Subsequently, methods were developed for synthesizing structurally simpler, more accessible analogues which retain high artemisinin-like activity. We describe our contributions to the design of new effective wholly synthetic trioxane candidates. *In vitro* and *in vivo* tests reveal that much of the skeleton of 2 is redundant and that, surprisingly, chirality is not required for activity. Discussion will focus on structure-activity relations and the search for the pharmacophore.

The possible mode of action of 1,2 and certain cis-fused tricyclic cyclopenteno-1,2,4-trioxanes (3) will be reviewed. They are believed to kill intraerythrocytic *Plasmodium* by a common mechanism which is different from that of chloroquine, namely, by interaction with the heme discarded by proteolysis of ingested hemoglobin. The first step entails complexation of heme to the peroxide bond. Thereafter, electron transfer generates an oxy radical that evolves to the ultimate parasiticidal agent. Two views prevail on the nature of this agent. It could be either an aggressive C-centered radical or Fe(IV)=O species. Each is thought to kill the parasite differently either by alkylating or oxidizing its membrane. Despite an understandable partiality for a P-450 type mechanism, it now appears that alkylation delivers the mortal blow.

It is concluded that this type of drug action can be conferred by a variety of peroxides, but ideally by a fluoro-derivative of 3. The latter has the merit of being cheaply available, non-toxic and effective. Therefore, it can be regarded as a possible replacement for chloroquine and artemisinin derivatives.

S - 25.6

SYNTHESIS AND ANTIMALARIAL ASSESSMENT OF A NEW GENERATION OF ANTIMALARIAL TRIOXANES

Chandan Singh, D Misra, G Saxena, N Srivastava, M Sharma, R. Kanchan, S Chandra, S K Puri, R Tripathi, G P Dutta and W K Milhous

Central Drug Research Institute, Lucknow 226 001, India.
Walter Reed Army Institute of Research, Washington, D C 20307-5100, U.S.A.

Artemisinin (Qinghaosu) and its derivatives are currently the most promising drugs for the treatment of chloroquine-resistant malaria. In our search for synthetic analogues of artemisinin, we have developed highly efficient methods for the preparation of cyclic peroxyketals. Following these methods a large number 1,2,4-trioxanes, trioxepanes and trioxocanes have been prepared. Several of these compound, particularly the 1,2,4-trioxanes, have shown very promising antimalarial activity both *in vitro* and *in vivo*. These trioxanes are structurally simple and can easily be prepared on multigram scale. Synthesis and structure activity relationship of these new antimalarials will presented.

S - 25.7

CHALLENGES IN MALARIA DIAGNOSIS

Wilbur K Milhous and Dennis E Kyle
Walter Reed Army Institute of Research, Washington, DC, USA.

There is an urgent and serious need for alternatives to microscopy for malaria diagnosis at all levels of the global health care system. In endemic areas where malaria is normally part of the differnetial diagnosis and where microscopists are trained and have adequate equipment, this poses few problems. With the increased prevalence of drug resistant strains, increased international travels and commerce along with changes in ecology and human demographics, there are more and more patients with unusual disease presentations in a variety of health care settings. Often malaria is not considered in the differential diagnosis and the results can be tragic. With the development of the new rapid and innovative diagnostic tests such as Parasight F and Optimal, significant advances have been achieved. These tests differ in terms of sensitivity and specificity as well as adaptability to patient management in contrast to diagnosis. The focus of initial this discussion will be to evaluate new methods to diagnosis malaria and to examine the role for these new technologies in routine malaria diagnosis and for patient management with special emphasis on strategies for aligning the goals of obtaining regulatory approval with global markets.

S - 25.8

α, β– ARTEETHER IN ACUTE UNCOMPLICATED FALCIPARUM MALARIA

N Valecha, S Gupta, Usha Devi, S Biswas, A Sharma, T Adak, O P Asthana and V P Sharma
Malaria Research Centre, 22-Sham Nath Marg, Delhi - 110 054.

Efficacy of α,β– arteether as an antimalarial was evaluated in Phase III clinical trial, conducted in 50 patients (42M + 8F) of acute uncomplicated falciparum malaria from

Delhi. Mean age was 27.3 years and mean parasitaemia on day 0 was 0.65%. Patients were treated with α,β– arteether at the dosage of 150 mg I/M for 3 days and followed up weekly for 28 days. With this treatment time taken for parasite clearance was 19.94 + 6.87 hours and fever clearance was 37.81 + 21.67 hours. 70% cases were fibril by 48 h and peripheral smear was negative in 100%. No side effects were reported. Recrudescence rate with this formulation was 6%, which is much lower than reported recrudescence rate for all other formulations of Artemisinin *viz.*, Artemether, Artesunate and Artemisinin. Our experience with Artesunate indicates high recrudescence rate of 93%. However, in this study carried out in 62 parients of uncomplicated malaria in Gujarat Artesunate was administered in very low dose for 2 days (240mg). It is concluded that arteether is safe, effective, rapidly acting antimalarial with low recrudescence rate.

S - 25.9

SUCCESS STORY OF aB, ARTEETHER, A NEW BLOOD SCHIZONTOCIDAL AGENT FOR MALARIA

O P Asthana,[1] J S Srivastava,[1] T K Pandey,[2] K A Vishwanthan,[2] Saroj Gupta,[3] N Valecha,[3] V K Srivastava,[4] P K Mahapatra,[4] J Mshsnts,[4] K M Mahapatra,[5] N C Nayak,[5] V Dev,[5] Neeru Singh,[6] M M Shukla,[6] A B Balsara,[7] B Dash,[8] S K Sathpathy,[8] S Mohsnty,[8] S Mishra,[8] V P Kamboj[1] and V P Sharma[3]

1. Central Drug Research Institute, Lucknow.
2. J.L.N. Hospital and Research Centre, Bhilai.
3. Malaria Research Centre & Lady Hardinge Medical College, New Delhi.
4. Regional Medical Research Centre, Dibrugarh.
5. CRPF Base Hospital & MRC, Guwahati.
6. Medical College & MRC, Jabalpur.
7. Tata Main Hospital, Jamshedpur.
8. Ispat General Hospital, Rourkela.

Arteether has been developed from Artemisinine, extracted from a Chinese plant *Artemesia annua. A. annua* has been successfully grown and cultivated by CIMAP Lucknow and a racemic mixture of a and B arteether (30:70) was supplied to CDRI for preclinical and clinical studies. Its safety and efficacy was proved in the preclinical, pharmacological and toxicity studies carried out in different animal models. I was found to be safe and effective fast acting blood schizontocidal agent. Phase I single dose (20-300mg IMI, OD) tolerance studies were undertaken in 30 normal healthy men and the multiple dose (100-150 mg IMI,OD*3 days) tolerance study was completed in 20 healthy subjects. All fifty volunteers tolerated the drug very well and no side effects of adverse drug reactions were encountered, In the phase II clinical trials carried out at Ispat General Hospital, Rourkela; Orissa, 49 patients of *P. falciparum* malaria were treated with the dose of 150 mg intramuscular injections of aB arteether given once a day on three consecutive days and were followed up for 4 weeks. In all the patients fever and parasitemia clearance was observed within the first week, recrudescence/reinfection

was seen in only 7 patients exhibiting a highly effective cure rate (86%). Phase III multicentric clinical trial with Arteether were conducted at 7 centres in 267 patients of uncomplicated and 211 patients of complicated *P. falciparum* malaria at 4 centres. The study was undertaken after obtaining clearance from the Drugs Controller (India) and Institutional Ethics Committee. Each patient was taken into this study after a written informed consent and was treated with a fixed dose schedule of Arteether given intramuscularly at a dose of 150mg once a day for 3 consecutive days. Tolerability to Arteether injection was good in all the cases and no significant adverse drug reactions were encountered during the trial. In uncomplicated group fever clearance time ranged between 24-144 hours, parasite clearance time was recorded in the range of 24-72 hours and 93-100% cure rate was observed in this study. In this group 236 (88%) patients out of 267 cases became afebrile within initial 72 hours. Rerudescence/reinfection found in this study was less than 7%. In complicated group fever clearance time ranged between 24-168 hours, parasite clearance time ranged between 24-120 hours with cure rate ranging from 92-100%. In this group 171 patients, out of 211 cases (81%) become fibril within first 96 hours. Overall mortality in this group ranged between 4 to 8.5% (14 out of 211 patients), of these 10 patients expired, within first 2 days before completing the 3 day schedule of Arteether therapy. These phase III multicentric clinical trial in 478 patients of *P. falciparum* malaria (267 uncomplicated and 211 complicated) established the efficacy of 3 day schedule of parenteral therapy of Arteether. Arteether has been licensed for marketing after obtaining necessary permission from the Drug Controller General (India).

S - 26.1

GENETIC SEXING STRAINS OF THE MEDFLY, *CERATITIS CAPITATA* : LESSONS FOR MOSQUITOES

A S Robinson
Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, IAEA Laboratories, A-2444 Seibersdorf, Austria.

Programmes to develop the Sterile Insect Technique (SIT) for mosquito control or eradication will be dependent on the generation of mosquito strains which enable large numbers of only male mosquitoes to be produced for irradiation and release. In fruit flies, genetic sexing strains have now been developed to a level which encbles them to be integrated into large scale SIT programmes. In fruit flies the elimination of males before release is mainly a matter of economy, although there is some evidence that released females although sterile can still oviposit in fruit and cause bacterial and fungal damage.

In the medfly, *Ceratitis capitata,* genetic sexing strains have been developed which enable either females to separated from males at the pupal stage due to a high temperature treatment. Both these types of genetic sexing rely on the use of male-linked translocations. By mapping the position of the relevant genes and the translocation breakpoints it has been possible to select combinations of marker and translocation which produce strains of remarkable stability.

The development of these strains will be described together with their integration into two large scale SIT programmes in Guatemala and Argentina. The Relevance and experience of these developments in medfly will be presented in relation to the selection of genetic sexing strains in mosquitoes.

S - 26.2

C B Beard

(Abstract not received)

S - 26.3

OVERVIEW OF REDUCED RISK TECHNOLOGIES FOR MANAGEMENT OF MALARIA VECTORS

S K Narang
National program Staff, U S Department of Agriculture, Agricultural Research Service, Beltsville, Maryland.

Pest control researchers and public health agencies are faced with a daunting challenge of finding effective environmentally benign methods for the control of insect vectors of

pathogens. Over the past three decades, many different control methods involving genetic and biologically-cased IPM approaches have been explored. Unfortunately, very few have been tested in field trials, and success has been limited. Many of these approaches are labor-intensive, cumbersome, and are not amenable to available or realistic formulation and delivery systems.

Novel approaches are being studies that use molecular biology and genetic engineering technologies. One fundamental goal is to transform a competent vector into an incompetent or a refractory vector for a specific pathogen. For example, blocking a critical site required for development of the pathogen in a vector would result in its inability to transmit the pathogen. Similarly, infection of a vector with a carrier virus expressing one or more antisense sequences to specific sequences of a virus pathogen may interfere with the replication and development of virus.

Recombinant sindbis virus has been successfully used by researchers at the Colorado State University, USA to make *Aedes aegypti* refractory to dengue virus. The use of molecular technologies provides a powerful tool and useful models for exploring similar approaches for malaria vector control. These and other similar approaches will be discussed.

S - 26.4

BIORATIONAL CONTROL OF MOSQUITOES

Norbert Becker
German Mosquito Control Association (KABS), Ludwigstr. 99, 67165 Waldsee, Germany

The discovery of toxin-producing mosquitocidal bacilli, such as *Bacillus thuringiensis* subsp. *israelensis* (Bti) in 1976 and potent strains of *Bacillus sphaericus* in recent years inaugurated a new chapter in the control of mosquitoes and black flies. The protection of humans against mosquitoes and mosquito-borne diseases e.g. malaria, lymphatic filariasis and dengue as well as the demand for the conservation of biodiversity and protection of nature by using target specific tools and appropriate control strategies can now be integrated by the use of biorational control measures against mosquito larvae. The rapid exploitation of bacterial control agents was aided by the relative ease with which they can be mass produced, their high efficacy, enormous environmental safety, ease of handling, relative low risk for the development of resistance, and their simple integration into control programmes involving community participation. However, higher costs compared to conventional insecticides and unsuitable formulations usually prohibit the widespread use of microbial control agents. The development of new tailor-made inexpensive and effective formulations against major nuisance and vector mosquitoes, which take the specific feeding behaviour of the target species and the most suitable mode of application in consideration, help to overcome these barriers in the use of microbial control agents. For instance, Culinex tablets, sporefree fizzy tablets which

are based on Bti and *B. sphaericus* allow a widespread and cost-effective control of container breeding mosquitoes particularly with community participation. Now a days, Bti is successfully used annually by hundreds of tons in many mosquito and black fly control programmes worldwide in temperate and tropical climatic zones. Due to its potential to persist and recycle in polluted water *B. sphaericus* is the most promising biocidal candidate for controlling larvae of *Culex quinquefasciatus*, the main vector of lymphatic filariasis. In recent large-scale field trials in India. Cameroon, Brazil, Sri Lanka and Tanzania, a remarkable impact of *B. sphaericus* treatments has been observed in reducing vector biting density by 80% through bi-monthly treatment of the mosquito larval habitats.

S - 26.5

MALARIA CONTROL WITH "ICON' (LAMBDA-CYHALOTHRIN)

G B White
Zeneca Public Health, Fernhurst, Haslemere, Surrey GU273JE, UK

Effective vector control is the first line of defence against malaria. Pyrethroid insecticides are the most powerful and cost-effective weapons for vector control. Lambda-cyhalothrin ("ICON") is the pyrethroid chosen by many authorities in Africa, Asia and the Americas for improved impact of vector control operations. House-spraying ICON 10% WP at rates of 25-30 mg/m^2 controls indoor resting Anopheles for 6-12 months. By introducing ICON for indoor residual spraying, countries such as Indonesia, Nepal, Oman, Sri Lanka, Vietnam, Zimbabwe and Latin America have improved coverage rates because ICON gives popular control of domestic pests (bedbugs, cockroaches, fleas, etc.) as well as long-term reduction of malaria transmission. Thus by using ICON instead of DDT or OPs, antimalaria campaigns have cut malaria incidence by 50-90% in problem foci. Also bednets impregnated with lambda-cyhalothrin 10-15 mg/m^2 provide good personal protection against malaria vectors for more than a year, and the treatment withstands washing. In West Africa and South America, for example, malaria incidence fell by >50% for people using ICON-treated bednets. Aqueous ICON CS (capsule suspension) formulation has optimal efficacy and user safety for treatment of bednets and curtains. ICON CS is supplied in sachets for treatment of individual bednets and in 'community packs' suitable for villages. Lambda-cyhalothrin was evaluated by WHOPES during the 1980s. Now marketed worldwide as ICON for vector control, this pyrethroid is effective against all malaria vector species, including DDT-resistant strains. Advantages of ICON include its rapid knock-down and kill of mosquitoes and other domestic pests, excellent residuality/dose ratio, no bad smell or staining, special unit packs and outstanding operational success record for malaria control.

S - 28.1

SELECTIVE, INTEGRATED VECTOR CONTROL FOR MALARIA

Pushpa R J Herath
Malaria Unit, Control of Tropical Diseases, World Health Organization, Geneva, Switizerland.

The discovery of the mosquito's role in malaria transmission has prompted anti-vector activities based, over the years, on a variety of methods. Experience cautions against over-reliance and non-discriminatory use of any one method, and supports a selective, integrated approach to vector control. This entails rational and cost effective use of control methods, alone or in combination, taking into account the focal and variable nature of malaria transmission and the absence of broad spectrum methods applicable to all situations.

Vector control methods include indoor residual spraying, insecticide-treated bednets and other personal protection measures, environmental management chemical larviciding, biological control and space spraying. Decision related to vector control depend on disease epidemiology, parasite/vector/human host characteristics, transmission patterns, the perceived social and economic consequences of malaria in affected areas, and the environment of transmission, The available resources and infrastructure for delivery are critical. Decision are guided by analysis of situations and information, generally at macro-level, and ideally also at micro-level for more closer targeting of interventions. The size of operational areas depends on the methods used and the levels of stratification and selectivity. Vector control may be targeted to i)address urgent malaria problems such as epidemics, high rates of mortality and morbidity, drug resistance, ii)prevent epidemics, and iii)reduce environmental risk of transmission, thus contributing to improvement of living conditions, health and development. Input requirements and implementing partners vary with the control target.

Efficacy and cost need close monitoring. When methods with different levels of efficacy and resource requirements are used, the relative contribution of each method needs to be regularly assessed. Management must ensure, adequate surveillance and information systems to recognize and pinpoint vector control needs, access to data from within and outside health services, timely and efficacious interventions, and logistic support. The technical staff must also be able to address the epidemiological aspects of transmission, the broader environmental issues involved in transmission risks, and community and intersectoral actions. Supporting policy frameworks must address i)the place of vector control in malaria control through decentralized general health services,ii)vector resistance management and the rational use of insecticides, iii) Import duty/tax exceptions and the use of quality assured products, and iv) safeguards against creation of mosquito genic conditions in development activities.
The experiences and literature on large-scale implementation of selective vector control are limited, and need expansion through appropriate control action in different epide-

miological settings and institutional arrangements, with careful documentation of the experiences.

A closely targeted, selective use of the available vector control methods can considerably reduce the health impact and social and economic burden of malaria.

S - 28.2

THE EFFICACY OF TREATED NETS IN VARIOUS EPIDEMIOLOGICAL SITUATIONS AND IMPLEMENTATION IN VARIOUS SOCIO-ECONOMIC SETTINGS

Jo Lines
DFID Malaria Programme, London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT.

The effectiveness of insecticide-treated nets is expected to depend, among other factors, on the behaviour of the vector. Adequate trials are still lacking for some important vector species. Nevertheless, epidemiological efficacy has been demonstrated against a wide variety of vectors, from the anthropophilic and late biting *An gambiae*, to the zoophilic and early-biting *An anthropophagus* and *An. albimanus*.

Effectiveness might also be expected to depend on the intensity transmission. Treated nets have nevertheless been shown to be effective in a wide range of epidemiological settings, from hypo-endemic parts of China to hyper-endemic parts of Africa. There remains doubt as to their long term benefits in areas with hyper- and holo-endemic transmission, a question which depends on the nature of natural immunity. The evidence available so far is not encouraging , but it is far from conclusive. It is not enough to reject the simple expectation that people who are less exposed to malaria are generally healthier than those who are more exposed.

The central obstacle to implementation, so far, has been the lack of a clear answer to the question "who will pay?". One simple possibility is that users pay for nets, and the government, with donor support, pays for the insecticide. This is the obvious choice where there is already a high rate of net usage, and where house-spraying is common. Unfortunately, the world's most malarious countries are also some of the poorest, and in many places, net use is rare and public health infrastructure is weak, especially in remote areas. In such places, public health funding must be focussed on providing for those most at risk; otherwise it is likely to be dissipated on marginal subsidies that benefit people who already have nearly enough money to pay for themselves.

S - 28.3

SELECTIVE VECTOR CONTROL OF MALARIA IN KARNATAKA, INDIA

S K Ghosh[1], V P Sharma[2], S N Tiwari[1], A K Kulshrestha[1], T S Sathyanarayan[1], R R Sampath[1], K J Ravindran[1], K Ravi Kumar[3] and M V Murugendrappa[4].
1. Malaria Research Centre, Bangalore.
2. Malaria Research Centre, Delhi.
3. Regional Office for Health & Family Welfare, Govt. of India, Bangalore.
4. Directorate of Health Services, Govt. of Karnataka, Bangalore.

Malaria was posing a serious problem in district Hassan, Karnataka. Two primary health centres (PHCs) Banavara and Kanakatte were taken for selective vector control of malaria. Extensive geographical reconnaissance in 160 villages under the PHCs revealed that major breeding sites for vectors were wells, tanks and streams and escape channels. *Anopheles culicifacies* was the main malaria vector followed by *An. fluviatilis*, Larvivorous fish Guppy (*Lebistes reticulates*) introduced in all the breeding sites specially in wells from March to August, 1996. In PHC Banavara (population 54, 749) only Guppy was used as intervention method. There was an overall reduction of about 54.5% in malaria incidence when compared with 1995 data. The effect of fish was more pronounced from September onwards and during this period an overall reduction of 78.3% was observed. In PHC Kanakatte three groups of villages were taken up. These were villages with i). Cyfluthrin impregnated bed nets (population 3448), ii), with both impregnated bed nets and fish (population 4651), iii). Guppy fishes (population 20,548). The reduction in malaria incidence in 1996 as compared to 1995 in the three areas were 81.8%, 70.3% and 68.3% respectively. Monitoring of entomological parameters in the above mentioned areas also showed a positive correlation with the reduction in malaria incidence. This study shows that use of larvivorous fish would be the most cost effective and sustainable method of malaria control in this area.

S - 28.4

'RECOGNISING THE SOCIAL PARADIGM' IN MALARIA CONTROL BEYOND TECHNO-MANAGERIALISM

Ravi Narayan
Society for Community Health Awareness, Research and Action, Bangalore - 560 034, Karnataka, India.

This paper derives framework and conceptual perspective from three processes in which the author has been involved in the context of malaria control.

• An Independent expert group process that reviewed the malaria situation and malaria control in India to derive issues of concern and alternatives for action from April 1996 to February 1997.

• A study- reflection-action-experiment focused on increasing involvement of voluntary agencies in Malaria control in Karnataka.

• A longer process of action-reflection with community health action initiators in South India for over 12 years through a catalyst technical resource cell which led to the conceptual evolution of a paradigm shift in action at grassroots level.

The paper outlines socio -cultural-economic-political-behavioural issues relevant to the malaria situation and the community at risk and makes a case for an urgent policy research shift from the agent -vector-environment paradigm to the societal context of policy and community context of programmatic action.

A case is made that the resurgence of malaria is not only due to agent resistance, vector resistance or other techno-managerial limitations and constraints but a deeper public health and development crisis and a growing health care system resistance that can be understood only by a increasing shift from molecular biology to a more grassroots socio -epidemiology.

Bringing back community empowerment to the core of the malaria programme is suggested as a major task ahead. This can be done by recognising the 'Social Paradigm' which will involve shifts of emphasis from individual cases to community problem; from patients as beneficiaries to people as participants; from drugs / vaccine / technology to knowledge transfer and social processes; from professional control to demystification; from centralised topdown national health programmes to decentralised local action strategies.

S - 28.5

INFORMATION MANAGEMENT IN VECTOR CONTROL

A R Wikremasinghe
Department of Community Medicine and Family Medicine, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Vector control is an integral component of malaria control. Vector control strategies have changed over time with the currently advocated one being selective. Vector control operations are very extensive and have to be carefully planned, implemented, monitored and evaluated. For each of these steps a substantial amount of data/information is required and has to process in an efficient way that would provide timely results. The validity of the results obtained from the ensuing analyses of this information will depend to a large extent on the quality, i.e. the reliability and validity of the data collected.

Being a selective operation it is important that the correct areas such as those having a high incidence of malaria, a high incidence of *falciparum* malaria especially drug resistance situations and those having the potential for malaria outbreaks etc. are identified

for implementation of vector control programs. Therefore, it is essential that reliable for valid baseline measures are available for planning effective and efficient services, and implementing, monitoring and evaluating such services. In addition to the frequently used parameters such as the incidence of malaria and the Pv:Pf ratio, other indices such as entomological parameters like vector abundance is useful in the ideal situation. in situations where additional information is not available proxy measures such as climate data for entomological parameters that are routinely collected by Departments of Meteorology can be used.

Information from sectors other than the health sector, which usually have their own information systems is necessary for planning and implementing vector control programs. Therefore, for optimal utilisation of information without unnecessary duplication, there should be close collaboration between all sectors. A common spatial unit that can be used by all sectors should be decided upon.

Valid and reliable data alone are not sufficient for developing an efficient and effective program. The data has to be processed and analysed to give rise to a decision support system that will provide relevant information in a timely manner. Newer information systems such as GIS and mapping programs can be used for this purpose.

S - 28.6

B G J Knols
(Abstract not received)

S - 29.1

PATHOGENESIS OF NEUROLOGICAL DAMAGE IN AFRICAN CHILDREN WITH FALCIPARUM MALARIA

Charles R J C Newton
Neurosciences Unit, Institute of Child Health, London, UK.

Plasmodium falciparum may be one of the most common causes of acquired neurological impairment in children living in sub-Saharan Africa. In contrast to non-immune adults with cerebral malaria, about 11% African children have severe neurological sequelae. Hamiparesis, spastic quadriparesis, visual impairment, language difficulties, ataxia and epilepsy are the most commonly described sequelae. The mechanisms of neurological damage are unknown and are likely to be different from those that cause death. In African children, neurological sequelae are associated with prolonged coma, seizures (particularly multiple or prolonged), hypoglycaemia severe anaemia and raised intracranial pressure, but a causal relationship between these factors and neurological damage is not established. The cause of coma in malaria is unknown, but impaired cerebral oxygenation and toxins such as cytokines, reactive oxygen species or nitric oxide may contribute to coma and thus sequelae. Seizures and hypoglycaemia are thought to cause damage by causing a mismatch between cerebral blood flow and metabolism, or by excitotoxins. Seizures, however, may be a manifestation of underlying neurological insult, or aggravate other causes of damage. Perturbations of cerebral haemodynamics may also contribute, although establishing these mechanisms as a primary cause is difficult. Neurological damage may also be caused by reactive oxygen species during the clearance of parasitaemia. Other mechanisms include, impaired mitochondrial function or apoptosis, both of which can be induced by cytokines. The relevance of these mechanisms to falciparum malaria will be discussed.

S - 29.2

CYTOADHERENCE CHARACTERISTICS OF *P. FLACIPARUM* INFECTED ERYTHROCYTES FROM MALAWIAN CHILDREN

Rogerson S J, Tembenu R, Dobano C, Molyneux ME, Taylor TE
Malaria Project and Wellcome Centre, Queen Elizabeth Central Hospital, Blantyre, Malawi

We have investigated the adherence of *P. flaciparum* infected erythrocytes (IE) isolated form Malawian children with severe or uncomplicated malaria to the receptors CD36, ICAM-1, chondroitin sulfate A (CSA) and thrombomodulin (TM) and to uninfected eythrocytes (rosette formation). IE from 52 children with severe malaria (30 cerebral malaria (CM) 12 CM and severe anaemia (SMA) and 10 (SMA) and 38 children with uncomplicated malaria were cultured successfully to trophozoite stage. Signicant binding (> 5 PRBC per mm2, at a standardised parasitaemia of 1%) to one or more receptors was seen in 61 of 83 isolates (75.3%), of which 59 bound to CD36 . Nineteen (21.9%)

bound to ICAM-1, seven to CSA and five to TM also. Mean binding to CD36 was significantly hier than binding to any other receptor. Approximately a quarter of isolates examined bound to none of the receptors examined. Only three isolates did not form rosettes (two from children with severe malaria) and mean rosetting rates were similar between groups (17.8 vs 14.7%).

When binding was compared between groups, there was significantly less binding to CD36 by isolates from children with severe malaria than isolates from controls (53±101 per mm^2 vs 245±452 per mm^2, p=0.02) but no other differences between groups. There was no association between rosette formation and disease severity.

S - 29.3

MALARIAL ILLNESS AND TOLERANCE : TWO SIDES OF THE SAME COIN.

Ian A Clark[1], Ian G Scragg[2] and Fadwa Al Yaman[1]
1. Division of Biochemistry and Molecular Biology, Australian National University, Canberra, Australia.
2. Department of Paediatrics, University of Oxford, John Radcliffe Hospital, Oxford, U.K.

Malarial illness and malaria tolerance provide good examples of the complexity involved in understanding the various roles nitric oxide can play in disease. As occurs with recurrent malarial infection, repeated exposure to bacterial endotoxin allows an individual to tolerate high doses without illness, a state referred to as endotoxin tolerance. Since human malarial infection generates tolerance to the harmful effects of both malarial parasites and bacterial endotoxin, we have addressed the possibility that tolerance to each agent might, as with their acute pathology, share a common mechanism. Endotoxin tolerance is now appreciated to be an active process, with enhanced iNOS and therefore nitric oxide production downregulating the generation of the pro inflammatory cytokines, such as TNF. This renders the host tolerant to endotoxin.

As with cytokines and malaria some years ago, literature is now appearing on the production of nitric oxide in this disease. Nitric oxide is a very short-lived mediator, with a correspondingly small sphere of influence, and the functional outcome of its generation depends on the proximity and nature of susceptible target molecules. One intriguing finding, reminiscent of the literature on endotoxin tolerance, is that total levels of nitric oxide (as indicated by serum nitrate levels) are highest during malaria tolerance, when TNF levels are lowest. While the functional implications of any overall increase in production of nitric oxide, as detected by raised serum levels of nitrate, cannot be ascertained without knowing its anatomical origins, it is possible to address the tolerance issue *in vitro*.

Using a model widely employed for investigating endotoxin tolerance, we have ex-

posed mouse peritoneal macrophages twice, 24 hr apart, to endotoxin-free malarial toxin extracted (Scragg et al. manuscript submitted) from *P. falciparum*. Reagents and media were treated with polymyxin B-coated beads. A second exposure to malarial toxin drove TNF production down dramatically. This decrease was accompanied by, and shown to be dependent in part on, an increase in nitric oxide. These *in vitro* findings suggest that total nitric oxide production (as indicated by serum nitrate levels) is high in the asymptomatic parasitaemia (ie malarial tolerant) children in Tanzania, Papua New Guinea, and Gobon because it is part of the mechanism that drives TNF levels down, thus causing malarial tolerance. The ability of nitric oxide to enhance I kappa B, the endogenous inhibitor of NF kappa B, which is the nuclear factor required for TNF to be generated, provides a plausible mechanism for this to occur.

S - 29.4

ANTIGENIC VARIATION AND CYTOADHERENCE OF MALARIA INFECTED CELLS

Grahan V Brown, John C Reeder, Kathleen M Davern, Stephen I Rogerson, and Alan F Cowman
The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Austrialia

Our laboratory has had a long standing interest in antigens expressed at surface of erythroctyes infected with *Plasmodium falciparum*. We demonstrated that antigenic variation occurs *in vitro* and that the variant antigen ifs *P. falciparum* erythrocyte membrane protein 1 (PfEMP1)[1]. Furthermore we demonstrated an association between phenotype and antigenicity, giving rise to a hypothesis of association between adherence to specific receptors and antigenicity, such that a limited number of variant antigenic types could give rise to severe pathology. [A direct link between cytokines and cytoadherence was demonstrated by Schofield and coworkers who showed that GPI (glycosylphosphatidylinositol) increases the adherence of malaia infected cells to endothelium, presumably by upreating specific receptors on the endothelial cells recognized by *P. falciparum* infected cells [2]]

Subsequent to the report of the structure of the *var* genes, our laboratory has worked towards cloning and sequencing a number of *var* genes. In particular we have focussed on attempts on identify and express the gene coding for a bindign motif for chondroitin sulfate A that has been shown to be an important binding receptor for *P. falciparum*[3].

In this session, further results on PfEMP1 will be presented andiscussed in relation to the possible roles in the area of pathogenesis.

S - 29.5

IMMUNOPATHOLOGY OF THE NEUROVASCULAR LESION IN CEREBRAL MALARIA : EXPERIMENTAL AND CLINICAL DATA

Georges E Grau[1], Giampaolo Pizzolato[1], Mireille Redard[1], Horst Bluethman[2], Malcolm E Molyneux[3] and Terrie E Taylor[3]
1. University of Geneva, Switzerland.
2. F. Hoffmann - la Roche, Basel1 Switzerland.
3. Malaria Research Unit, MRC, Blantyre, Malawi.

Tumour necrosis factor (TNF) has been implicated in the pathogenesis of experimental cerebral malaria (CM), but the respective role of its two types of receptors has not been established. Upon infection with *Plasmodium berghei* ANKA, a significant increase in the expression of TNF - receptor 2 (TNFR2, p75), but not of TNFR1 (p55), was found on brain microvessels at the time of CM in susceptible animals. Moreover, mice genetically deficient for TNFR 2 (Tnfr 2 = B0) were significantly protected from experimental CM in contrast to TNFR1-deficient (Tnfr1 = B0) mice, that were as susceptible as wild type mice. Interestingly, the pronounced ICAM-1 upregulation and leucocyte sequestration, typically occurring in the brain microvessels of CM-susceptible animals, was detected in infected control and Tnfr1=B0 mice, whereas no such ICAM-1 upregulation nor leucocyte sequestration were observed in Tnfr2=B0 mice. Using cultures of brain microvascular endothelial cell (MVEC) isolated from wild type, Tnfr1=B0 or Tnfr2 =B0 mice, we showed that membrane - bound TNF only needs TNFR2 for TNF-mediated ICAM-1 up-regulation, while soluble TNF requires both receptors. Solely in MVEC lacking TNFR2, neither membrane-bound nor soluble TNF upregulated ICAM-1 *in vitro*. In conclusion, the interaction between membrane TNF and TNFR2 is crucial in the development of this neurological syndrome. The possible involvement of TNF receptors and of other cell surface molecules was studied on multiple-site brain samples from Malawian children who died of severe *P. falciparum* malaria. Immunohistochemical analysis revealed a marked upregulation of TNFR2, but not of TNFR1, in several cell types : endothelial cells, with a preponderance on the abluminal side as well as some neurones, pericytes and microglial cells. An analysis of the correlation between these changes and the clinical and biological parameters will be presented.

S - 29.6

IMBALANCED DISTRIBUTION OF *PLASMODIUM FALCIPARUM* MSP-1 ALLELES RELATED TO SICKLE CELL TRAIT

Odile Mercereau-puijalon[1], Francine Ntoumi[1,3], Christophe Rogier [2], Helene Jouin,[1] Alioune Dieye[2], Pascal Millet[3] and Jean-Francois Trape[4]
1. Unite d' Immunologie Molecular des Parasites, CNRS U1960, Institut Pasteur, Paris.
2. Institut Pasteur, Dakar, Senegal.
3. International Centre for Medical Research, Franceville, Gabon.
4. Laboratoire de Paludologie, ORSTOM, Dakar, Senegal.

Analysis of isolates collected from asymptomatic carriers living in Dielmo, (a holoendemic Senegalese village) indicated that the distribution of the 3 MSP -1 allelic families and of individual MSP-1 alleles was influenced by age and differed in AA and AS subjects. There was no influence of other genetic characters or of location of residence within the village. Distribution of MSP-2 alleles was only marginally affected. In another endemic area with different ethnic groups, transmission intensity and parasite populations, the influence of sickle cell trait on the distribution of parasite types was confirmed in a single age group; a similar imbalance in MSP-1 allele distribution associated with the sickle cell trait was observed in a cohort of Gabonese school children.

The data suggest that the haemoglobin type imposes some constraint on the characteristics of the parasites hosted, reflecting a pressure on MSP-1 itself or on a linked locus. Several, non-mutually exclusive mechanisms could contribute to an imbalanced strain distribution related to host haemoglobin type: selective immune clearance of parasites presenting certain alleles, strain differences in capacity to complete intraerythrocytic development and/or premature elimination of infected cells, resulting from impaired cytoadherence in AA or AS cells. Analysis of the allele specific immune response will be discussed.

S - 29.7

B D Das
(Abstract not received)

S - 30.1

THE NATURAL HISTORY OF HIV - 1/2 INFECTION IN MUMBAI

Subhash K Hira
AIDS Research & Control Centre (ARCON), J J Hospital, Mumbai & the University of Texas-Houston, USA

OBJECTIVE : 1) To determine the natural history of HIV-1/2 in Mumbai ; 2) To determine role of concomitant tropical infections.
METHODS : 3583 patients referred to ARCON Centre at JJ Hospital for features suggestive of immune suppression were recruited into the study between April 1994 and January 1997. They were offered HIV test after counselling. Those testing HIV positive on two ELISA systems were folowe dlongitudinally every 3 months. All relevent laboratory investigations were performed to diagnose opportunistic infections. They were staged as Asymptomatic. AIDS-related complex and AIDS using NACO definition.
RESULTS : The clincial spectrum of HIV-associated illnesses at recruitment was :

Symptom/Disease	HIV+	HIV-	OR CI.95
Fever > 1mo	n=1842 (%)	n=1741 (%)	
Wt Los > 10%	440 (22.2)	78 (4.5)	6.1 (4, 7, 7, 9)
Pulmonary TB	407 (22.1)	98 (5.6)	4.7 (3, 7, 6, 0)
Cough > 1mo	303 (16.6)	90 (5.8)	3.7 (2, 9, 4, 8)
Oral Candida	160 (8.7)	78 (4.5)	4.1 (3, 1, 5, 3)
PGL	159 (8.6)	17 (1.0)	9.7 (5, 7, 16, 5)
Herpes zoster	155 (8.4)	40 (2.3)	4.0 (2, 8, 6, 8)
Diarrhea > 1mo	131 (7.1)	35 (2.0)	4.5 (3, 0, 6, 6)
Extra-Pul TB	63 (3.4)	6 (0.3)	10.2 (4,2, 26.3)
CNS Toxoplasma	30 (1.6)		

Among 520 patients followed for 781 person years, the incidence of disease progression from Asymptomatic to AIDS was 8/100 p-y. The overall rate of mortality was 7/100 p-y. The survival curve showed that 50% of asymptomatic HIV+ developed AIDS in 7 years.
CONCLUSIONS : The rate of disease progression oas compared with US/European Cohorts, is twice faster among heterosexual HIV-infected population in Mumbai. The role of tuberculsis and other tropical infections (including malaria) in accelerating the disease progression has emerged in this study.

S - 30.2

INTERACTION BETWEEN HIV AND MALARIA IN PREGNANT WOMEN IN ENDEMIC AREAS

R W Steketee, B L Nahlen, M E Parise and L J Schultz

In Africa, HIV-1 is the most serious emerging infectious disease and *Plasmodium falciparum* malaria is one of the most prevalent infectious diseases. In some endemic areas of sub-Saharan African, more than 50% of women have malaria infection during pregnancy, and more than 30% of women of child bearing age are infected with HIV-1. Most studies suggest that there is no biologic link between the two; that is, malaria has not appeared as an opportunistic infection, nor does it speed the progression of HIV-1 disease. However, altered cell-mediated immunity in HIV-1 infected persons could influence the frequency and course of malaria infection. In Africa, pregnant women, who are already more susceptible to malaria infection than their non-pregnant counterparts, may be vulnerable to the immune system effects of HIV-1 infection,either during the first pregnancy, in which malaria susceptibility is greatest, or in subsequent pregnancies in which immune recognition of malaria parasites must be established and maintained. To evaluate this potential interaction, we examined the impact of HIV-1 infection on malaria in pregnant women in rural settings of sub-Saharan Africa. We examined the association between HIV-1 and *P. falciparum* in pregnant women enrolled in a malaria chemoprophylaxis study in rural Malawi. All studies were approved by human subjects review committees of participating organizations. Pregnant women in rural Malawi, western Kenya, and coastal Kenya were evaluated at their first antenatal clinic visit (mean 5-6 months of pregnancy) and either followed through delivery (in Malawi) or resurveyed in a cross-sectional study at delivery (Kenya sites). *P. falciparum* parasitemia was measured at antenatal visit, at delivery, and postpartum (Malawi only); placental and newborn (umbilical cord blood) infection was measured for hospital-delivered infants. Serum collected during pregnancy was tested for antibodies to HIV-1 by enzyme immunoassay with Western blot confirmation. In Malawi, parasitemia was detected in 46% of 2946 women at enrollment and 19.15 at delivery; HIV seroprevalence was 5.5%. The prevalence and geometric (GMPD) of parasitemia at enrollment and at delivery were higher in HIV-seropositive(+) than in HIV-seronegative (-) women (at enrollment: 57% prevalence and GMPD of 1558 parasites/mm^3 *vs* 44% and 670/mm^3, respectively, $p<0.0001$; and at delivery: 35% and 1589/mm^3 *vs* 18% and 373/mm^3, $p<0.0005$) . Placental infection rates were higher in HIV(+) compared with HIV(-) women (38% *vs* 23%, $p<0.0005$). Similar findings were observed in Kenya. Compared with infants born to HIV(-) women, newborns born to HIV(+) women had higher rates of umbilical cord blood parasitemia. HIV(+) and HIV(-) women had similar rates of parasitemia postpartum. HIV -1 infection diminishes a pregnant women's capacity to control *P. falciparum* parasitemia and placental and newborn infection, the major determinants of *P. falciparum's* impacts on fetal growth and infant survival.-

S - 30.3

B Nahlen
(Abstract not received)

S - 30.4

HIV TRANSMISSION BY BLOOD TRANSFUSION FOR THE TREATMENT OF MALARIAL ANAEMIA: A PREVENTABLE INFECTION

Carlos C Campbell
University of Arizona Prevention Center, Tucson AZ USA. and Eve M Lackritz[1]
Centers for Disease Control and Prevention, Atlanta GA USA.

Concurrent with the evolution of the global HIV epidemic, resistance in *Plasmodium falciparum* to available drugs has become more prevalent. Resistance to chloroquine in Africa has become intense, with enormous impact on the clinical manifestations of malaria in children and other at risk populations. Beginning in the mid-1980s *Plasmodium* infections treated with chloroquine persisted and were more frequently associated with severe anaemia (SA-Hgb< 5 gm%) in East and Central Africa. In hospital mortality rates of SA exceeded 20% in many areas, with the highest incidence in children < 2 years of age; blood transfusion rates for hospitalized patients have escalated in malarious areas. Surveys in East and Central Africa documented that blood transfusion constitutes a common mode of HIV transmission. In many malarious areas the lack of blood banking, poorly applied or lack of HIV screening tests, as well as the rapidly increasing prevalence of HIV infection in the population, have created a crisis in blood safety. Efforts, some successful, at implementing blood screening and banking, and to limit transfusion to only those patients who will benefit have been implemented in some areas.

Effective malaria therapy must include the prevention of SA which in turn minimizes the risk of exposure to blood products. Malaria therapy policies must stress the need for parasiticidal drugs particularly for young children in areas with malaria-associated SA. Progress in deployment of single test, rapid screening for HIV depends on financial and governmental commitment to securing blood for patients with life-threatening anaemia.

S - 30.5

MALARIA INFECTION AS A CO-FACTOR IN HIV INFECTION AND REPLICATION

Altaf A Lal
Division of Parasitic Diseases, National Centre for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Ga-30341.

Over half of the HIV-1 infections in the world occur in sub-Saharan Africa. More than

90% of the world's 300-500 annual malaria cases also occur in the region. In addition, circumstantial evidence indicates that progression from HIV infection to AIDS in malaria-endemic areas may be substantially faster than in nonmalarious regions. Because malaria infection activates mononuclear cells and stimulates the production of proinflammatory cytokines, a possibility exists that malaria infection may promote HIV-1 infection and replication. In order to understand the interaction between malaria antigens on *in vitro* infection of unstimulated peripheral blood mononuclear cells (PBMC) with HIV-1. Stimulations with both soluble malarial antigens and purified malarial pigment significantly enhanced HIV-1 replication in CD8-depleted PBMC from both CCR-5 +/+ as well as CCR -5-/- (homozygote for 32 bp deletion) donors, when compared with untreated PBMC. The stimulatory effect was malarial antigen specific, since control erythrocyte antigens or proteinase-treated pigment did not result in enhancement of HIV-1 replication. Both malarial preparations mitogenically activated PBMCs from healthy donors as judged by the expression of cell activation markers, proliferative responses, the cytokine production. Furthermore, neutralizing mAb to TNF - α inhibited malarial antigen or pigment-induced HIV-1 replication, whereas neutralizing mAb to IL-6 had no significant effect. Malarial preparations also increase HIV-1 replication by activating LTR-directed viral transcription. The results of this *in vitro* study demonstrate a profound modulation of HIV replication by malarial antigens, and suggest that malaria infection can modulate HIV-1 pathogenesis both by promoting omoting the infection through the activation of lymphocytes, and by increasing viral replication through the production of cytokines. We caution, however, that interpreting this data to link malaria with rapid emergence of AIDS be held in abeyance until field-based immunoepidemiologic investigations are conducted to address this issue.

S - 31.1

DIVERSITY IN PLANT AND SOIL NEMATODES (NEMATODA) OF RAJASTHAN (INDIA) : GAPS IN RESEARCH

Qaiser H Baqri
Zoological Survey of India, Desert Regional Station, Jodhpur

Amongst the different ecological groups of the phylum Nematoda, the plant and soil nematodes play important role in every ecosystem. The information provided hereunder on these nematodes from Rajasthan is based on nematology literature and identification of species made by the author during the last two years.

Arya (1957) was the first to report a *Meloidogyne* species infesting tomato crop in Jodhpur district of Rajasthan. The important references on survey and identification of plant and soil nematodes of Rajasthan include Prasad, Mathur & Sehgal (1959), Khera and his co-workers (1966-75), Yadav and his co-workers (1966-80), Swarup & Sethi (1968-71), Mulk & Jairajpuri (1975-76), Nama and his co-workers (1981-91), Sethi & Swarup (1986), Lal & Mathur (1986), Sharma & Trivedi (11993) and a few others.

In all, 115 species belonging to the Orders Tylenchida, Aphelenchida, Dorylaimida, Rhabditida, Monhysterida and Enoplida had been reported up to 1996. Out of these, 38 species were described as new to science. After identification of a nematode collection by the present author, 29 more species of the Orders Tylenchida and Dorylaimida are being added as new records from Rajasthan. As such, 144 species have been identified till date. Of these, about 70 species are phytophagous belonging to the Orders Tylenchida, Aphelenchida and Dorylaimida while remaining are either predaceous or saprophagous.

The family wise information on economically important phytophagous nematodes can be summarised as follows: Tylenchidae (2spp.), Psilenchidae (3 spp.), Dolichodoridae (10 spp.), Hoplolaimidae (13 spp.), Heteroderidae (4 spp.), Meloidogynidae (2 spp.), Pratylenchidae (12 spp.), Tylenchulidae (1 sp.), Rotylenchulidae (1 sp.), Anguinidae (5 spp.), Paurodontidae (3 spp.) Aphelenchoididae (8 spp.), Longidoridae (2 spp.) and Xiphinematidae (4 spp.). The important and widely distributed nematodes are : *Tylenchorhynchus mashhoodi, T. brassicae, Quinisulcius capitatus, Hoplolaimus indicus, Helicotylenchus dihystera, Pratylenchus thornei, P. scribneri, Heterodera avenae, H. cajani, H. sacchari, H. zeae, Meloidogyne incognita, M. javanica, Tylenchulus semipenetrans, Rotylenchulus reniformis, Anguina tritici, Xiphinema basiri* and *X. indicum*. The report of "ear-cockle" and "Molya" diseases in wheat, barley and millet caused by *Anguina tritici* and *Heterodera avenae* respectively, may be considered as important finding of survey and identification work in the state. Among the predaceous and saprophagous groups, the maximum species (35) have been recorded of the Order Dorylaimida whereas the Order Mononchida is being represented only by one species in the entire state.

The review of nematology literature reveals that nearly 9.5% of plant and soil nematode fauna of India has been reported from Rajasthan whereas the state occupies about 10.3% area of country. Though Rajasthan appears to be a significantly surveyed for plant and soil nematodes, the state still considered to be a poorly surveyed. This observation can easily be supported from the fact that nearly 60 species (4% nematode fauna of India) have been reported from Delhi which occupies only 0.004% area of the country. In fact, the significantly surveyed districts like Ajmer, Bikaner, Chittorgarh, Jaipur, Jodhpur, Nagaur, Pali and Udaipur should also be considered as partially surveyed districts. The identification of economically important groups like criconematids, trichodorids and mono- chids has been neglected in Rajasthan. The information on the degree of dominance and frequency of occurrence of phytophagous nematodes is also insignificant, except a few districts. The record of only 41 species of dorylaimids, including six as parasitic, cannot be considered as significant.

The survey and identification of plant and soil nematodes in the command area of Indira Gandhi Canal should be completed at the earliest so that the baseline data can be prepared before minor pests may assume the status of major pests in the changed ecological conditions, as reported in insects.

S - 31.2

QUANTITATIVE DISTRIBUTION OF NEMATODES ON PASTURE OF LITAN AND COMPARISON BETWEEN THE NEMATODES FAUNA OF TWO CLIMATICALLY DIFFERENT AREAS

N Mohilal, Ch Dhanachand
Parasitology Laboratory, Life Science Department, Manipur University, Canchipur, Manipur

Quantitative distribution of three groups of nematodes - Tylenchs, Dorylaims and Mononchs are recorded in three different pastures i.e. unutilized pasture, utilized pasture and cattle fold. The result reveals that the Tylenchs have almost similar number in both unutilized and utilized pastures while in cattle fold their number decreases. Dorylaims have the highest population in cattle fold while the predatory nematodes are more in unutilized pastures.

A survey to determine the percentage frequency of occurrence for both phytophagous and soil nematodes around the roots of *Cynodon dactylon* pers of Ukhrul and Chandel Districts of Manipur revealed *Helicotylenchus, Aqlenchus, Axonchium, Iotonchus, Parahadronchus* as a few among the most populated and *Boleodorus, Psilenchus, Pratylenchus, Hirschmanniella, Discocriconemella, Oriverutus* as among the least populated ones. The population of *Pratylenchus, Macroposthonia, Discocriconemella* are more in Chandel district while *Tylenchorhynchus, Axonchium* and *Oriverutus* are more in Ukhrul district. The lowest index of similarity is in between Chakpikarong and Moreh while the highest index of similarity is in between Ukhrul and Litan.

S - 31.3

INFLUENCE OF PARASITISM ON THE LIFE CYCLE PATTERNS OF PHYTONEMATODES

Harish K Bajaj
Department of Nematology, CCS Haryana Agricultural University, Hisar - 125 004, India.

Among root parasitic tylenchids there are interrelated evolutionary trends towards endoparasitism often accompanied by a host response in the vicinity of stylet, swelling of females and production of large number of eggs. Such trends have resulted in the diversification of life cycle patterns of sedentary ecto/endoparasitic nematodes from simple life cycle of ectoparasites. Sedentary nematodes evolved several strategies for the completion of their life cycles which included : 1. Defining of infective stage - J_2 or/and female, 2. Lengthening of duration of J_2, 3. Retention of cuticles of preceding stages thereby making J_3 and J_4 non feeding stages, 4. Development of males through metamorphosis, 5. Retention of eggs inside the female body and 6. Secretion of gelatinous matrix. Different groups of nematodes have adopted different routes in their life cycles using these strategies. Route A- Female infective stage; development from J_2 to adult in soil; J_3 and J_4 inside the cuticle of preceding stages J_2 infective stage, female development inside the roots. Route C- J_2 infective stage, female and male development on to the roots as sedentary ectoparasite; male development direct, entering the soil at J_4 stage; J_3 and J_4 female retaining cuticle of preceding stages only in the posterior body region. Route D- J_2 infective stage; male development by metamorphosis, inside the roots. Subroute D_1- J_3 and J_4 with stylet; tendency towards the retention of eggs. Subroute D_2- Duration of J_2 very long; J_3 and J_4 of short duration, enclosed within cuticles of preceding stages. Route E- J_2 infective stage, male development by metamorphosis, J_3 and J_4 ?; preparasitic vermiform female in soil, reinfecting roots. Life cycles of all the genera of Heteroderidae when fully known have helped in the better understanding of phylogeny of the family.

S - 31.4

SOME STUDIES ON PREDATORY SOIL NEMATODES (MONONCHIDA : NEMATODA) FROM ALPINE-ZONE OF GARHWAL HIMALAYAS, UTTAR PRADESH INDIA

Vikram Singh Rawat, Nisha Srivastava and Maqsood Ahmad
Deptt. of Zoology, Govt. P.G. College, Rishikesh-249 201, U.P. India.

A survey of predatory soil nematodes from alpine-zone (above 3000 M.high) in two districts of Garhwal Himalayas, U.P., India yielded about 120 populations of predatory nematodes. The frequency of these nematodes belonging to the order Mononchida Jairajpuri, 1969 was recorded as 58%. In Chamoli district, the alpine-zone areas were Chandrashila, Tungnath, Chopta, Hemkund, Valley of Flowers (Nanda Devi Biosphere Reserve) and Auli, while Gangotri, Yamnotri and Gaumukh places are in Uttarkashi

district. The eight genera of predatory nematodes belonging to the order Monochida Jairajpuri, 1969 were collected, *Viz., Mylonchulus, Prionchulus, Clarkus, Coomansus, Mononchus, Sporonchulus* and *Miconchus*. Among these frequencies of the genus *Mylonchulus* was highest (25%). Three species were recorded from these areas, *viz., M. amurus, M. minor* and *M. lacustris,* these have been identified and described. Two populations were designated as new species. Similarly the genus *Prionchulus* was collected in 21 samples (Frequency = 17.5%) and *P. muscorum* was the most frequent. Morphometric and allometric variations of this species have been studied in detail. A population was found different from all *P. muscorum* populations and it is proposed here as a new species. The frequencies of *Clarkus, Coomansus, Iotonchus, Sporonchulus, Mononchus* and *Miconchus* were 21.00, 17.00, 3,00, 2.5, 1.00 and 1.00% respectively. Showing that few species of order Mononchida are residents of alpine areas in Garhwal Himalaya, U.P., India.

S - 31.5

NEW AND KNOWN NYGOLAIMOID NEMATODES FROM GARHWAL HIMALAYAS, UTTAR PRADESH INDIA.

Maqsood Ahmad
Department of Zoology, Govt. P.G. College, Rishikesh - 249 201, U.P., India.

During 1994-96, about 300 soil samples were collected from Garhwal Himalayas, U.P., India for the recovery of plant-parasitic and soil nematodes. These yielded several known and five new species of soil nematodes belonging to the superfamily Nygolaimoidea Thorne, 1935. The known species were identified as *Clavicaudoides clavicaudatus, Aquatides deconincki, A. intermedius, Laevides laevis, L. timmi, Solididens vulgaris, Paravulvus acuticaudatus, P. teres, Aetholaimus indicus, Nygellus subclavatus.* These have been measured and described briefly. *Paravulvus teres* description based on males and females has been reported here for the first time from India. Five new species, two of the genus *Paravulvus* Heyns, 1968 and one each in the genera *Clavicaudoides* Heyns, 1968, *Aetholaimus* Williams, 1962 and *Aporcelaimoides* Heyns, 1965 have been proposed. All of these have been measured, illustrated, diagnosed and described in detail.

S - 31.6

ADAPTIVE RADIATIONS IN THE FEEDING APPARATUS OF DORYLAIM NEMATODES

Wasim Ahmad
Section of Nematology, Department of Zoology, Aligarh Muslim University, Aligarh 202 002, India

In soil, the nematodes dominate in number as well as in species over all other soil-inhabiting animals collectively; and have occupied all possible habitats representing a very wide range of biological diversity. Several taxonomic groups of nematodes in-

habit soil but the members of the order Dorylaimida occur more frequently than any other group. They are found world over, in all types of soil and in fresh water; in every conceivable habitat and usually dominate both in number and species over all soil inhabiting nematodes. It is not uncommon to find 8-10, some times more Dorylaim species representing as many genera in a single soil sample.

Over 2000 known species of Dorylaimida which constitute about 1/10 of the total known nematode fauna exhibit considerable diversity in their size, structure of feeding apparatus including oesophagus, tail shape and reproductive and copulatory structures. The highly diversified feeding apparatus is indicative of their varied feeding habits and occurrence in diverse habitats. The entire lip region including lips and papillae exhibit considerable modifications and is directly correlated with the feeding habit. The structure of vestibule, guiding ring, odontostyle, odontophore and oesophagus show a very high degree of adaptive divergence and definite lines of gradual modification (evolution) could be traced for both predatory and parasitic mode of life.

The phytoparasitic and predatory mode of feeding appear to have evolved several times in this group as is evident from the superfamilies Dorylaimoidea and Tylencholaimoidea.

NOTES

ORAL SESSION

O - 1.1

CHARACTERISATION OF TWO GENES PRESENT IN A REGION HIGHLY CONSERVED IN DIFFERENT *PLASMODIUM* SPECIES

Marta Ponzi, Cecilla Birago, Leonardo Picci, Tomasino Pace.
Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy.

A 10kb genomic region, highly conserved from rodent to human conserved from rodent to human Plasmodia, has been analysed. It contains two transcription units running in Opposite directions and separated by an intergenic region of a few hundreds base pairs. One of the two genes encodes a nuclear factor possibly involved in nucleosome assembly, as suggested by its similarity to yeast, mouse and human Nucleosome Assembly Proteins. Monoclonal antibodies raised against its expression product clearly reveal the nuclear localisation of the protein both in *P. berghei* and in *P. falciparum.*

The second gene identified a 4kb mRNA in *P. berghei* and the deduced protein (113kD) exhibits significant blocks of similarity with the γ-glutamyl-cysteine synthetase (γ-GCS) heavy subunit. This enzyme is involved in the biosynthesis of glutathione (GSH) whose function is crucial in oxidant protection of the cell.

The comparison with the *P. falciparum* counterpart reveals regions with 80% identity and short, less conserved regions in which identity drops to 15%. Interestingly, the *P.berghei* and *P. falciparum* ORFs are longer than genes encoding the γ-GCS-heavy subunit from other sources. Domains specific of the parasite absent in mammalian enzymes can be identified.

The presence of a *Plasmodium* γ-GCS counterpart transcribed during the erythrocytic cycle represents the first evidence for the presence of endogenous synthesis of glutation, suggesting that the parasite is not dependent from the erythrocyte GSH pool. Possible mechanisms of control of γ-GCS expression are discussed.

O - 1.2

CLONING SEQUENCE ANALAYSIS AND EXPRESSION OF ERYTHROCYTE BINDING PROTEIN FROM *PLASMODIUM CYNOMOLGI BASTIANELLI*

Pawan Malhotra, Daniel M.N. Okenu, P.V. Lalitha, Chetan Chitnis and V.S. Chauhan
International Centre For Genetic Engineering and Biotechnology, New Delhi, India

The invasion of human erythrocytes by plasmodial merozoites involves a multi step process that is mediated by specific molecular interactions between the merozoites and the target erythrocytes. Erythrocytes binding proteins (EBP) which include the Duffy binding proteins (DBP) of *P. vivax* and *P. knowlesi* and 175 kDa sialic acid binding protein of *P. falciparum,* have been shown to be involved in this interactions. We have cloned a member of erythrocyte binding protein family from *P. cynomolgi.* It shares

structural similarities with other members of the same family. Comparison of amino acid sequence of *P. cynomolgi* EBP with EBP of *P. vivax, P. kowlesi* and *P. falciparum* shows that *Pc* EBP is most similar of *P. vivax* DBP and least similar to the *P. falciparum, P. vivax* and *P. knowlesi* to be involved in erythrocyte binding. Based on the homology it is predicted that the binding domain of *Pc* EBP will also lie in region II. We have expressed *Pc* EBP region II in *E. coli* as well as in COS cells. Studies are in progress to assess its erythrocytes binding characteristics.

O - 1.3

HETEROGENEITY IN HEAT SHOCK PROTEIN GENES IN *LEISHMANIA* ISOLATES

Sunil K Arora, G S Kapoor and S Sehgal
Department of Immunopathology, Postgraduate Institute of Medical Education & Research, Chandigarh - 160 012, India.

Leishmaniasis, a group of visceral and cutaneous diseases, are caused by parasites belonging to one genus comprising of about 13 different species. Many methods including serological, biochemical and molecular biological techniques have been used by various workers to characterize these different species and isolates of *Leishmania,* yet there is no single generally accepted criterion. We have identified certain cDNA clones from the library generated from the promastigotes of S1 strain of *Leishmania donovani* and used them as probes for identification of various isolates of *L.donovani* and *L. tropica.* Two of the probes used, E2b (2.0kb) and Ela (1.3 kb), sequence characterized to be hsp70 of *Leishmania,* were able to distinguish various isolates of *L. donovani* from different geographical origins as well as strains of *L. donovani* from those of *L. tropica.* Thus, the recombinant hsp 70 cDNA probes could be a potential molecular marker for characterization of leishmanial strains as well as geographical variants.

O - 1.4

MALARIAL HSP WITH METALLOPROTEASE MOTIF

Y D Sharma, J M Fakruddin
Department of Biotechnology, All India Institute of Medical Sciences, New Delhi, India.

Earlier, we have isolated immunodominant clones from the *Plasmodium vivax* - DNA library in Lambda g/11. One of the clone, named Pv9, is being described here. The complete gene was isolated and sequenced. The deduced amino acid sequence of Pv9 gene, not the nucleotide sequence, showed a significant homology to the small heat-shock protein 'HtpX' of *E.coli.* The Pv9 gene in parasite was also found heat inducible thus we concluded that it is a heat shock protein gene. The Pv9 gene encodes for 483 amino acids of calculated mol. wt.. 51.22 kDa. The protein was found to be expressed at all the blood stages of *P.vivax,* based on immunofluorescence assay. The anti Pv9 anti-

body, however, recognized three bands: ~55 kDa, 30 kDa and 28 kDa. However, the 28 kDa band was found to possess the protease activity which was predicted from the HEXXH sequence motif present in Pv9. Therefore, the ~55 kDa band is a proenzyme and it is processed to the mature form of 28 kDa with 30 kDa as inactive propeptide. We, therefore, conclude that we have isolated the unique heat-shock protein of *P.vivax* with protease activity.

O - 1.5

NEW INTERSPECIES CONSERVED OF *PLASMODIUM FALCIPARUM*

F A S Kironde, N Sahoo, D Ma and S Paliwal

The results of earlier and more recent malarial vaccine trials, in which individual or cocktails of polypeptides from proclaimed "vaccine candidates" including RESA, CSP, MSA1 and others did not induce satisfactory immune protection calls for a careful re-assessment. Failure of the trials could be due to improper delivery of the vaccine assessment. Alternatively, it may be that we do not know the correct immunogens or antigen combinations to vaccinate with. Regardless, it is now imperative that we should encourage the application of different novel approaches towards identifying new vaccine candidates. Our research group has applied a number of such unique approaches. We have isolated rhoptries of *P. falciparum* merozoites and raised rhoptry specific antiserum that may be used as immunoscreen probe for indentifying new rhoptry antigens. We estimated more than 40 polypeptides to be present in lanes of SDS-PAGE analysis of the rhoptry fraction, confirming that a greater majority of the rhoptry antigens have not been cloned and sequneced. Significantly, most of the rhoptry polypeptides appear to be recognized by human anti-*P. falciparum* sera, raising the question why only about 5 rhoptry antigens have been identified and characterized so far. We assume that the polypeptides detected in our rhoptry fractions represent distinct antigens and are not degradation products of a feq rhoptry proteins. Equally interesting, we found that a smaller but considerable number of rhoptry antigens are recognized by either mouse anti *P. yoelii* sera or rabbit antiserum (anti-IMP serum) raised against putative transmembrane proteins of *P. falcoparum*. These and other initial data from analysis of the rhoptry fraction, hyperimmune antisera to the faction and other fractions of merozoites will be discussed. Earlier, by co-probing *P. falciparum* and *P. yoelii* and is probably a transmembrane molecule. We have recently ascertained that a 34-kDa fragment of this apical antigen induces partial inhibition of the growth of *P. yoelii* and *P. falciparum*. These results will be presented. Further, still using the co-probing approach, we identified another new antigen designated pf60 that is also shared between *P. yoelii* and *P. falciparum* Interestingly, the shared epitopes of pf60 appear to lie on a 7-kDa polypeptide fragment that contains s zinc-finger like motif. Cloning and expression of the zinc-finger like polupeptied will be discussed.

O - 1.6

C Qin
(Abstract not received)

O - 1.7

ALLELE-SPECIFIC PCR FOR DIAGNOSIS OF DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM:* OPTIMIZATION OF PCR CYCLES

Ravi Toteja, V K Bhasin
Department of Zoology, University of Delhi, Delhi 110 007, India

All known DNA polymerases elongate the chain from base - paired ends, and mismatch at the 3' - end is unfavoured for this elongation reaction. Allele-specific PCR uses this phenomenon to detect presence or absence of previously characterized point mutation(s) in the target DNA. The base exchange primarily at the position 323 of the gene coding for dihydrofolate reductase (DHFR-TS) of *P. falciparum* is known to impart pyrimethamine resistance in these parasites. Accordingly, two 15 mer diagnostic primers and one 21mer common primer were designed from DHFR-TS gene sequence and custom synthesized. The PCR cycles were optimized for these primers to discriminate between the DNA of pyrimethamine sensitive and resistant falciparum lines. However, at times it was difficult to amplify even the small DNA fragment of 350 bp under standard conditions. This was perhaps due to high A+T rich nature of *P. falciparum* genome. We have found that reduction of PCR extension temperature from 72°C to 60°C circumvents this problem. In the first amplification step a lager DNA fragment was amplified using this modified temperature followed by the stringent PCR conditions. Thus, the primers employed were found to be very effective in discriminating DNA of pyrimethamine sensitive and resistant parasites.

O - 1.8

EXPRESSION OF MEROZOITE SURFACE ANTIGEN-2 (MSA-2) OF *PLASMODIUM FALCIPARUM* IN BACULOVIRUS VECTOR.

Pramatha R. Bhattacharya,[1] Mukesh Kumar,[2] Vijay P.S. Rawat,[2] Parvinder K. Rathee[2] and Rakha H. Das[2]
1. Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.
2. Centre for Biochemical Technology, Mall Road, Delhi University Campus, Delhi-110 007, India.

Merozoite surface antigen-2 (MSA-2) of *Plasmodium falciparum* is a potential candidate for vaccine development. That is why it needs to be expressed in large quantity. MSA-2 gene has been cloned and expressed in *Escherichia coli* and yeast. But the expression has not been efficient and very successful in these systems. So we have cloned the MSA-2 gene of *P.falciparum* in pCR script SK (+) vector and sequenced. The MSA-2 gene has been excised from the vector and cloned into Baculovirus expres-

sion vector and *Spodoptera leutera*, cell lines were infected. SDS-PAGE of the proteins showed very good expression of MSA-2 gene.

O - 1.9

MOTILITY OF THE *PLASMODIUM FALCIPARUM* MEROZOITE-THE ROLE OF CYTOSKELETAL PROTEINS AND THEIR LOCALISATION.

R E Fowler[1], L H Bannister[1], J C Pinder[2], F M Lavin[1], F A Mir[1], R E Fookes[1], A R Dluzewski[2], W B Gratzer[2] and G H Mitchell[1].
1. Dept. of Immunology and Dept. of Anatomy and Cell Biology, UMDS, The Medical School, Guy's Hospital, London Bridge, SE1 9RT.
2. Muscle and Cell Motility Unit. The Randall Institute, King's College, London, 26-29 Drury Lane, London, WC2B 5RL.

Malaria merozoites actively enter red blood cells (rbc) probably using an acto-myosin motor. We have previously demonstrated the presence of myosin and filamentous actin in the merozoite. Using antibodies raised against *P.falciparum* actin and myosin, we have evidence from immunofluorescent and immunogold staining that these proteins are located on the membranes and in the apical region of the merozoite. We have used toxins and inhibitors which act on these proteins to elucidate their role in the invasion process.

We have previously demonstrated that merozoite tubulin is important for successful invasion of rbc's. We have been investigating what this role might be. Using toxins with known effects on tubulin, we have shown that the integrity of merozoite microtubules was important for successful invasion, but that stabilising microtubules had no effect. We will also present evidence that some aspects of organelle distribution may be affected by drugs which act on parasitic tubulin.

O - 1.10

IDENTIFICATION OF MINIMAL CD36-BINDING DOMAIN WITHIN SEQUESTRIN, A *PLASMODIUM FLACIPARUM* PROTEIN INVOLVED IN CYTOADHERENCE

Anjali Yadava and CF Ockenhouse
Department of Immunology, WRAIR, Washington DC.

Pathology related to cerebral malaria in humans is associated with the binding of parasitized erythrocytes to the endothelial linings of the brain capillaries, a phenomenon known as cytoadherence. This process is mediated through specific receptor-ligand interaction(s). While several host molecules have been shown to serve as receptor(s), only two proteins have been identified as parasite ligands on the surface of infected erthrocytes. One of these molecules is a > 200 kD, antigenically conserved protein called sequestrin, which has been shown to mediate parasite binding to the endothelial receptor, CD36. The partial gene encoding for sequenstrin has been cloned and sequenced.

A putative CD36 binding domain comprising of a region spanning 139 amino acids has been previously identified within the partial sequestrin gene and referred to as R01. Congenic mice immunized with recombinant GST-R01 induced antibodies which inhibited the binding of parasitized erythrocytes to CD36. In an attempt to further delineate the CD36-binding domain of sequestrin, we have made a series of deletion constructs of R01 and expressed the recombinant proteins in *E. coli*. This approach has allowed us to precisely map the CD36 binding domain within a region of 62 amino acids. Using site-directed mutagenesis, we are in the process of identifying the crucial amino acids within this region which participate in binding to CD36 on the host cell. These studies will enable us to further understand the molecular basis of sequestration/ cytoadherence and design a more effective vaccine which will prevent cytoadherence.

O - 1.11

CIRCUMSPOROZOITE PROTEIN (CSP) POLYMORPHISM IN *P. VIVAX* ISOLATES OF INDIA

Hema Joshi and T Adak
Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054

Isolates collected from subjects having *P. vivax* infection were analyzed by polymerase chain reaction (PCR) for variations in CSP. PCR amplified product was further analyzed by hybridizing with allele specific oligonucleotide probes using digoxigenin labelling method. Results indicated the presence of CS I and CS II variants of CSP among *P. vivax* isolates collected from Delhi. A good proportion of isolates showed the presence of both the alleles thus suggesting presence of more than one clone in the isolate. These results substantiate our earlier reports on multiclonal nature of *P. vivax* isolates in India.

O - 1.12

SELECTION OF A GENOTYPE OF *PLASMODIUM FALCIPARUM* DIHYDROPTEROATE SYNTHETASE BY PYRIMETHAMINE-SULFADOXINE TREATMENT

Jill Curtis, M T Duraisingh, D C Warhurst and C F Curtis
Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, UK

Blood samples from Tanzania, collected before and 3 weeks after treatment with pyrimethamine-sulfadoxine (PSD) were analyzed for variants of the genes for dihydropteroate synthetase (DHPS) and dihydrofolate reductase (DHFR) of *P. falciparum* which have been associated with *in vitro* drug resistance. There was *in vivo* selection of a specific genotype, ser436, gly437, glu540 of DHPS by PSD treatment. A combination of chlorproguanil and dapsone used in parallel, did not select for the DHPS variants present in the local population. Both drug combinations selected for ile51,

arg59 and asn108 of DHFR.

O - 1.13

MALARIA DURING PREGNANCY IN TRIBAL BELT OF CENTRAL INDIA.

Neeru Singh[1] and V P Sharma[2]
1. Malaria Research Centre (Field Station), Medical College Building, Jabalpur - 482003
2. Malaria Research Centre, 20, Madhuvan, Delhi - 110 092

It is generally agreed that pregnant women are especially prone to severe attacks of malaria which may cause abortion, premature labour and still birth. Only a few hospital based studies carried out in different parts of India provide limited information. Therefore, this study was undertaken (1996-1997) in tribal belt of Central India, an area of perennial malaria to elucidate the prevalence of malaria in pregnant women to define some of the effects that it may exert during pregnancy to provide information for the development of malaria control strategy for this high risk group.

In all 137 pregnant women and 140 nonpregnant women were selected randomly in 10 tribal villages of Narayanganj PHC of Distt. Mandla during active transmission period and followed them longitudinally for the duration of pregnancy and puerperium. Slide positivity rate was over 70% in pregnant women and 50% in nonpregnant women. *Plasmodium falciparum* was the most prevalent species accounting for 90% of total malaria infection in both pregnant and nonpregnant women. There were no instances of cerebral malaria or death, however abortions, still birth and anaemia were commonly prevalent in most of the women (63%). Malaria parasites were not found in 25 neonates born to mothers with heavy parasitaemia at the time of delivery. However, two neonate were found positive for *P. falciparum* on day 10 and day 18 after birth.

Failure to clear *P.falciparum* parasitaemia after chloroquine administration (25mg/kg of body weight) was common. Persistent parasitaemia was recorded in 30% pregnant women while only in 19% of nonpregnant women ($p < 0.001$). Poor response to chloroquine suggests the need to change the drug policy.

O - 1.14

POTENTIAL NOVEL TARGETS IDENTIFIED BY GENE AMPLIFICATION IN LEISHMANIA

R Madhubala, Anirban Ghosh, Vandana Dhanjal, V Samuel Raj and Kenneth Stuart
School of Life Science, Jawaharlal Nehru University, New Delhi and Seattle Biomedical Research Institute, Seattle, USA

Leishmania are protozoan parasites that cause Leishmaniasis, a spectrum of diseases dependent on species involved. Leishmania species are responsible for morbidity and

mortality in many areas of the world, Major outbreaks of Visceral Leishmaniasis have occurred in India, Bangladesh, Brazil and Sudan. Leishmanial patients refractoriness to antimonial therapy is a rapidly growing problem. Thus, there is an urgent need for alternative approaches such as development of effective vaccine or diagnostic for drug resistance. The key to developing an effective vaccine against Leishmaniasis is dependent on identifying the most appropriate molecular targets. The LD1 DNA is proposed to be one such target DNA which is amplified in a variety of forms at high frequency, suggesting that it has an important role. Many Leishmania isolates have electrophoretic karyotype indicative of amplification of sequences from a locus on 2.2 Mb chromosome. About 5% isolates have 100 copies/cell of a 54.4 Kb circular molecule composed of an inverted dimeric repeat of a 27.2Kb sequence (that we call LD!) derived from the 2.2 Mb chromosome. Another 14% of isolates contain 10-50 copies/cell of 220-550 Kb linear chromosome that contain all or part of the LD1 sequence. An additional 3% of isolates contain different type of amplification, implying that all amplifications are not yet discovered. Database searches with nucleic acid sequences predicted that LD1 has nine potential genes (A--1) transcribed from the same DNA strand, apparently as polycistronic transcription unit. Database searches have identified some of these genes : B encodes ribosomal protein L37, C has a homology with SfhB and other bacterial gene. H encodes a small basic, potential ribosomal protein and I encodes a protein with a nucleotide binding motif. The amino acid sequence predicted from G contains 10-12 potential membrane spanning remains implying that it may be a transporter. It also has a homology to ESAG 10 from the VSG expression site to *T.brucei*. A, D, E, F appear to be newly described genes. The LD1 sequence and its organization, including that of flanking sequences, is conserved in numerous *Leishmania* isolates. Interestingly, it appears that the F and G fenes are tha only ones amplified LD1 sequences. We are presently working on the functions of these genes of LD1 and possible relationship between their amplification and drug resistance is being explored. We have presently cloned or fF gene jin a bacterial expression vector pET-17b and thereof expressed as recombinant protein. Fusion protein with full length F was purified to immunize rabbits. Westem analysis predicted size (~34 KD) in the all *Leishmania* promastigotes tested. Increased expression of F protein was seen in the strain 51.1 which overexpresses F mRNA from the rRNA locus but there is little if any increases in strains with other amplification possibly reflecting the presence of antisense RNA complimentary to F mRNA. Preferential expression of F in patient isolates and amastigotes was also observed. The probable role of some of these genes will be discussed.

O - 1.15

EXPRESSION AND CHARACTERIZATION OF THE BINDING DOMAIN OF AN ERYTHROCYTE BINDING PROTEIN FROM PLASMODIUM VIVAX

Akash Ranjan, Sanjay Singh, Kailash Pandey and Chetan Chitnis.
Malaria Research Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asafali Marg, New Delhi , India.

The interaction of the erythrocyte binding proteins , such as the Duffy antigen binding proteins of *Plasmodium vivax* and *P. knowlesi*, and the sialic acid binding protein of *P.falciparum*, with their receptors on human erythrocytes plays a critical role in erythrocyte invasion. It has been previously shown that the functional binding domain of these proteins is localized to a conserved, approximately 350 amino acid long cysteine-rich region that is present in each protein. Based on the presence (usually 12 in number) of conserved cysteines and characteristic stretches of hydrophobic amino acid residues, these domains are classified as a family of domains that are known as the Duffy binding like (DBL) domains. We have expressed the erythrocyte binding domain of the Duffy antigen bringing protein of *Plasmodium vivax* as a soluble, secreted molecule in a mammalian cell expression system. This recombinant molecule has a 6-histidine tag fused to its C-terminus to facilitate direct purification by Ni-NTA chromatography.

We have also developed an assay to determine the binding of recombinant molecules to Duffy positive human erythrocytes. The recombinant molecules were shown to be functional in this assay. In order to map the binding site within the cysteine-rich domain, a panel of chimeric constructs containing regions of the *P. vivax* domain and the homologous domain from the *P. knowlesi* β protein were made. Erythrocyte binding assays with these chimeric molecules localised the binding site to a 100 amino acid long stretch between the fourth and seventh cysteines of the *P. vivax* binding domain.

O - 1.16

OVERCOMING *PLASMODIUM FALCIPARUM* CODON USAGE PROBLEMS IN E. COLI BY SUPPLEMENTATION OF RARE t-RNA MOLECULES

David E Lanar, Victoria L Miller, Coleen Martinez, Victor M De Armas and Meredith R Abbott
Department of Immucnlogy, Walter Reed Army Institute of Research, Washington DC 20307 USA

Growth of *P. falciparum* proteins in *E. coli* has been disappointing. Atttempts often have lead to bacterial cell death or poor levels of expression upon induction of the heterologous protein. One cause for this phenomenon is ogten attrrubuted to "poor codon usage" whereby the codon in the parasite gene for a particular amino acid has a rare or under-represented cognate t-RNA molecule available in the *E. coli* host. For example, the codons most often used in *P. falciparum* mRNA for the amino acid Arginine are AGG or AGA however the t-RNAs for these condons are very rarely found in *E. coli* which prefers to use the codons CGA or CGU. This problem is enhanced when high expression of a protein sequence containing rare codons is required. The most common method of overcoming this inconsistency is to change the *P. falciparum* cloned gene sequence by single point mutations or complete gene resynthesis to change the codon sequence but not the resultant amino acid. This can become problematic when many rare codons are involved. An alternative is to supplement the *E. coli* with plasmids that encode rare codons needed by the heterologous *P. falciparum* protein. We

have done this to dramatically increase the expression levels of the C-terminal portion of LSA-1 in *E. voli*. Without supplementation most protein synthesis was terminated prematurely and only small microgram quantities of full length product were recovered per liter of culture. After cotransfection the expressing *E. coli* host with a plasmid containing the *argU* gene encoding the rare t-RNA AGA/G production levels increase to over 8 mg/l. We have made plasmids containing the genes encoding other rare t-RNAs for Asn and Pro and are evaluating them in the expression of other malaria genes in *E. coli*. Results will be presented.

O - 1. 17

IN VITRO RECONSTRUCTION OF MALARIA PARASITE-DERIVED KNOBS FORMED ON THE SURFACE OF *PLASMODIUM FALCIPARUM*-INFECTED HUMAN ERYTHROCYTES

Athar H Chishti, H Derick, Charles E Bell and James Murray
Department of Biomedical Research, St. Elizabeth's Medical Centre, Tufts University School of Medicine, Boston, Massachusetts, USA.

A distinctive pathological feature of *Plasmodium falciparum* malaria, the most lethal form of human malaria is cytoadherence, the attachment of parasitized erythrocytes to microvascular endothelial cells. *P. falciparum*- infected erythrocytes display elytron-dense knob-link protrusions (termed knobs) on the cell surface, which act as attachment points in the cytoadherence of parasitized erythrocytes. Previously, we isolated and characterized knobs from *P. falciparum*-infected erythrocytes and found that parasite-encoded knob-associates histidine-rich protein (KAHRP) was a major component of purified knobs. In this study, we have demonstrated that recombinant KAHRP self-associates to form knob-like spiral-ring structures closely resembling the morphology of purified knobs. These findings suggest that KAHRP is a key structural component in the assembly of knobs. We have shown that KAHRP is anchored to spectrin-actin-protein 4.1 complexes through binding interactions with actin and spectrin. Moreover, we have found that knobs are localized to spectrin-actin-protein 4.1 junction in the spread membrane skeleton of *P. falciparum*-infected erythrocytes. These findings are consistent with our view that knobs are formed mainly by self-associating KAHRP anchored to spectrin-actin-protein 4.1 junctional complexes in the host cytoskeleton. Cytoadherence of *P. falciparum*-infected erythrocytes is attributed to parasite var gene products (termed VAR), a family of antigenicallyvarient receptors localized to surface knobs. Despite their high antigenic diversity, VAR proteins have a remarkably conserved cytoplasmic domain (termed VARCD). We have shown in this study that recombinant VARCD binds to KAHRP as well as actin, spectrin, and spectrin-actin-protein 4.1 complexes. These findings suggest that the localization of VAR to knobs is achieved through a binding interaction(s) between a highly conserved cytoplasmic domain of VAR and KAHRP. Binding interactions of VAR with host spectrin-actin-protein 4.1 complexes in the knob assembly may provide further stability to the antigenically-diverse receptor function of VAR.

O - 1.18

NUCLEIC ACID IMMUNIZATION WITH MALARIA SEXUAL STAGE GENES Pfg27 AND Pfa25

Cheryl-Ann lobo and Nirbhay Kumar
Dept of Molecular Microbiology and Immunology School of Public Health and Hygiene John Hopkins University 615 N. Wolfe Street, Baltimore, MD 21205

The 27kDa (Pfg27) and the 25kDa (Pfa25) antigens of *P.falciparum* sexual stages are both important targets of antibody mediated malaria transmission blocking immunity. We have studied the relative immunogenicity of these antigens when presented alone or in a hybrid gene construct, in the form of DNA based immunogens. We were interested in looking at changes in the immune response, both at the B and T cell level, when the antigens were presented together in the hybrid construct. We have therefore cloned the genes enconding Pfg27, Pfs25 and a molecular hybrid of the two, Pfg27-Pfg25 into the DNA vaccine vector VR1012 (interacellular expression) and VR1012 (secreted). Balb-c mice were immunized and boosted in groups of 5 by either intramuscular or intradermal routes. A comparison of the immune response, both humoral and cellular, elicited by the different fene constructs using the two different routes of immunization in the two DNA vectors against the target antigens will be presented. These results could have important implications in the design of more effective transmission blocking vaccines.

O - 2.1

TOTAL FREE AMINO ACIDS OF *MONOLIFORMIS DUBIUS*

Mrunalini Joshi[1] and R Suhasini[2]
1. P.G. Department of Zoology, A K Navjivan Women's College, Ramkote, Hyderabad.
2. Department of Zoology, Nizam College, Osmania University, Hyderabad-500 001.

Monoliformis dubius inhabits the small intestine of *Rattus rattus* (The common house rat). It is also reported in children and adult human beings. This parasite is known to cause extensive damage to the intestinal tissues because of its armed probocis, often causing minute perforations in cases of heavy infection. Lack of sufficient information with regard to some biochemical aspects of this parasite has led the authors to probe in this area. This paper deals with the free amino acids content of *M.dubius*. It has been found that free amino acids are more in male worms than in female worms. These results have been discussed in detail in the paper.

O - 2.2

DEVELOPMENTAL REGULATION OF PROTEIN KINASE C ACTIVITY AND INHIBITION BY ANTIMALARIAL DRUG CHLOROQUINE.

Arun Sharma and Sukla Biswas
Malaria Research Centre, 22 - Sham Nath Marg, Delhi- 110 054.

P. falciparum undergoes complex physiological changes during development from ring stage to schizont in the erythrocyte of mammalian host. Since calcium and phospholipid-dependent protein kinase C (PKC) has a central role in the regulation of this function, we have examined changes in PKC activity during parasite maturation. Activation of protein kinase C required Calcium, Phosphatidyl serine and either Deacylglycerol or phorbol esters. A nine fold increase in the activity of PKC was found in schizonts as compared with ring stage of mature schizonts was found to be associated with translocation of PKC activity from cytosolic to membrane fractions. We have also found that chloroquine directly inhibited PKC activity in a dose development manner in *P. falciparum* sensitive strains. However, in the resistant parasites, the activity is differentially expressed during parasite development and may have critical roles in regulation of cellular events in *P. falciparum*. Inhibition of PKC activity by antimalarial drug, chloroquine may provide new insights into the mechanism of chloroquine resistance.

O - 2.3

SERUM BIOCHEMICAL CHANGES IN FALCIPARUM MALARIA

Ramesh R Dhande; Deepa Dhande & G N Vankhede
P. G. Department of Zoology, Amravati University, Amravati, 444602 (India)

Forty patients with heavy infections of *Plasmodium falciparum* were investigated for

serum biochemical alterations. In all the patients, significant increased values of LDL + VLDL cholesterol ($P < 0.001$) and total cholesterol ($P < 0.01$) were observed alongwith highly significant low levels of HDL cholesterol. A decrease in total serum proteins mainly due to a decrease in serum albumin was seen.

Highly elevated levels of serum SGPT, alkaline phosphatase ($P < 0.001$) and lactate dehydrogenase ($P < 0.01$) were reported in all the patients. However, highly significant lowering in blood glucose and cholinesterase activity was noticed in all the cases under treatment. The serum urea and serum creatinine levels rose sharply to and above 140 mg/100 ml and 5.2 mg/100 ml respectively in all the malarial patients All the above results are indicative of liver dysfunction during falciparum malaria.

O - 2.4

ISOENZYMES : A TOOL TO STUDY SPECIES / STRAIN DIFFERENTIATION AMONGST PARASITE POPULATION

S T Pasha, P Sethi, D S Rawat and J Sokhey
Division of Biochemistry & Biotechnology, National Institute of Communicable Diseases, 22 - Shamnath Marg, Delhi - 110 054.

Isoenzymes being considered as stable characteristic in nature, have invariably been used as genetic markers to study the species / strain differentiation amongst various parasites. Technique of isoenzyme variation has been considerably used employing polyacrylamide gel, starch gel as media for electrophoresis. Variation in varied strains of malaria parasite has also been studied and significant differences have been found in isoenzymes pattern. Studies on variations have been carried out on *P. falciparum* from various countries mostly from African Region, using starch gel electrophoresis, which is very cumbersome and time consuming. The present paper describes the variation for LDH & GPI isoenzymes in various species of plasmodia namely, P. *gallinicium, P. berghei, P. cynomolgi, P. knowlesi* and compared with *P. falciparum.*

Blood from fowl, rat & rhesus monkey infected with *P. gallinicium, P. berghei, P. cynomolgi* and *P. Knowlesi* was collected in chilled citrate saline at peak parasitaemia. P. falciparum was grown *in vitro* and blood was collected when the parasite count was around 11 percent. After washing the infected blood with normal saline, the parasite was separated from RBCs with saponin lysis. The parasite thus, obtained was frozen and thawed at least 5 times. Electrophoresis was carried out in agarose at pH 8.6 at current of 7.5 mA per slide for two hours for both LDH & GPI isoenzymes. Staining for LDH and GPI was done by layering the specific staining solution on the slides. Results have clearly indicated that variation exists in isoenzyme patterns for both LDH & GPI amongst all the five different species of plasmodia.

O - 2.5

COMPARATIVE ENZYME PROFILE OF PATHOGENIC AND NON-PATHOGENIC STRAINS OF *LEISHMANIA*

Nazneen B Khan , W A Nizami
Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh-

202 002 (U.P.), INDIA.

The promastigote stages of a pathogenic strain (Dd8) and a non-pathogenic strain (UR6) of *L. donovani* were maintained under *in vitro* culture and the harvested promastigotes were used to study their enzyme profile and elucidate the differences, if any. The enzymes estimated were acid and alkaline phosphatase, Na+ K+ ATPase, 5' nucleotidase and γ-Glutamy1 transpeptidase.

Among the phosphomonoesterases, acid phosphatase, alkaline phosphatase and 5' nucleotidase activity was found to be more in the non-pathogenic strain as compared to the pathogenic strain. Another enzyme γ-GTP activity was also observed to be more in UR6 compared to Dd8. However, in contrast to these enzymes activities Na+ K+ ATPase was observed to be higher in Dd8 strain.

An average of 2-2.7 fold increase was observed in acid, alkaline phosphatase and γ-GTP levels in the non-pathogenic strain whereas 6 folds increase was observed in the 5' nucleotide activity in UR6 strain as compared to Dd8. Na+ K+ ATPase showed 1.87 folds enrichment in the pathogenic Dd8. It is possible that this enzyme Na+ K+ ATPase plays some role in the virulence mechanism of this strain Dd8.

O - 2.6

EFFECT OF *IN VIVO* TREATMENT WITH CHLOROQUINE (CQ) AND DIFLUOROMETHY-LORNITHINE ON POLYAMINE METABOLISM OF CQ-SUSCEPTIBLE AND RESISTANT *PLASMODIUM BERGHEI*

Bahu L Tekwani, Manjari Mishra and Subhash Chandra
Central Drug Research Institute, Lucknow-226 001 (India)

Polyamines play important role in cellular growth and proliferation. Inhibition of polyamine biosynthesis has been identified as a potential target for chemotherapy of protozoal infections including malaria. A sequential increase in the level of polyamines occurs during erythrocytic schizogony of malaria parasites. Ornithine decarboxylase (ODC), a regulatory enzyme of polyamine biosynthesis pathway, has been found to be a peak function enzyme. Chloroquine (CQ) causes potent inhibition of malarial ODC *in vitro*. ODC of malaria parasite is highly sensitive to inhibition with difluoromethyl Ornithine (DFMO), an active-site directed inhibitor of the enzyme. DFMO also shows potent antimalarial action *in vitro* on *Plasmodium falciparum* cultures. The ODC inhibitors however, do not show promising antimalarial efficacy under *in vivo* conditions. The effects of *in vivo* treatment with CQ and DFMO on polyamine biosynthesis of CQS and CQR *Plasmodium berghei* proliferating in *Mastomys coucha* was therefore studied. Spermidine was found to be the major polyamine of CQS *P. berghei* and putrescine and spermidine were present almost in equimolar concentrations. CQR *P. berghei* also showed similar polyamine profile, but the levels of all the three polyamines were marginally lower as compared to the CQS parasite. Activity of ODC also was almost

the same in both CQS and CQR parasites. The treatment of infected animals with a sub lethal dose of CQ (8 mg/Kg body wt. in a 3 day oral schedule) did now cause any effect on parasitaemia levels. The CQS parasite registered marked decrease in spermidine contents while putrescine and spermine levels remained almost unchanged due to the treatment. Polyamine profile of CQR *P. berghei* did not change due to treatment with CQ. The antimalarial action of CQ on the susceptible parasite *in vivo* is preceded with depletion of spermidine. CQ also inhibits uptake of polyamine from the extra cellular medium. Treatment of the infected animals with DFMO (1% in drinking water for 3 days) did not bring any change in parasitaemia of CQS as well as CQR *P. berghei* infected animals. Both CQS and CQR parasites registered low but significant depletion in spermidine contents which may not be sufficient to check the growth and proliferation of malaria parasites *in vivo*. The results thus, confirm the role of polyamine in growth of malaria parasites under *in vivo* conditions.

O - 2.7

PURIFICATION AND CHARACTERIZATION OF *S. CERVI* PROTEASES

S Rathour and R. N. Singh
Department of Biochemistry, Faculty of Science, B.H.U., Varanasi.

Literature reveals that a good deal of proteases secreted by different filarial worm. Though, these worms possess a very primitive alimentary canal, secretion of proteases by them indicates their roles in escape mechanism from host defence and generation of a portal of entry through host tissues they inhabit. Microfilarial stage of *S.Servi*, a bovine filarial parasite, secrete proteases on *in vitro* cultivation. Specific staining of enzyme in gel showed two bands of proteolysis. When purified by DEAE-Sepharose CL6B, one enzyme was found in unbound fraction and another was eluted with 0.1 M NaCl gradient. Further, two enzymes were purified by Sephadex G-100 column chromatography. The silver staining of SDS-PAGE of unbound enzyme showed single band at 50 KDa whereas two bands of molecular mass 70 KDa and 56 KDa were observed in bound fracton of which 70 KDa was protease. The pH optima of 50 KDa and 70 KDa was 5.2 and 7.0 respectively. Both enzymes exhibited collagenase activity. 70 KDa enzyme was significantly inhibited by serine group inhibitors and metal ion chelators while 50 KDa enzyme was inhibited by leupeptin, a thiol group inhibitor. DTT accelerated proteolysis to a large extent by 50 KDa enzyme supporting the role of thiol group in its activity. Both enzymes were inhibited by *W. bancrofti* infected sera.

O - 1.8

MOLECULAR CHARACTERIZATION AND LOCALIZATION OF *PLASMODIUM FALCIPARUM* HSP 60

A Das[1], C Syin and N Kumar
Molecular Microbiology and Immunology, johns Hopkins School of Public Helath, Baltimore, MD, Food & Drug Administration, Rockvilly, MD.

Heat shock proteins (Hsp) are a group of highley conserved proteins which are widely represented phylogenetically. These were originally identified as cellular proteins expressed at elevated levels in response to a cariety of stress stimuli or heat shock. Most Hsps are constitutively produced in cells and are essential for maintenance of normal cellular functions. The Hsp 70 and Hsp 90 of the malaria parasite *P. falciparum* were described earlier. Recently, the Hsp 60 analogue in *P. falciparum* Hsp 60 in *E. coli*. Antisera raised against the recombinant Hsp 60 was employed in immunoprecipitation studies with biosynthetically labeled parasite extracts to 3-4 fold accumulation of Hsp 60 transcripts in heat shocked parasites (37°C to 40°c), the expression of Hsp 60 was not induced in the blood stages of *P. falciparum*. In previous studies with Hsp 70 on the other hand, the effect of heat induction was seen both at the level of mRNA and protein. In these studies we also observed co-immunoprecipitation of a number of other cellular proteins suggesting possible interaction with Hsp 60. In addition, IFA analysis indicated that the protein was localized predominantly in the cytoplasm in all the various stages of the parasite. These studies suggest that different mechanisms are involved in the regulation of expression of various members of the heat shock proteins in the parasite.

O - 3.1

MECHANISM OF ANAEMIA IN FALCIPARUM MALARIA

Bhabani S Das
Department of Biochemistry, Ispat General Hospital, Rourkela - 769 005, Orissa, INDIA

Logically, the genesis of anaemia in *P. falciparum* malaria could be because of augmented erythrocyte destruction and bone marrow dysfunction. Both these mechanisms were examined. There was a remarkable decrease in plasma haptoglobin and an increase in total and unconjugated bilirubin indicating significant haemolysis. Marrow imprint and biopsy showed a normally responsive marrow, with no conspicuous dyserythropoisis. Depleted marrow iron reserve indicated preexistent iron deficiency.

Augmented destruction could be possible because of erythrolysis of infected cells, oxidative damage to RBCs, and enhanced pitting of RBCs in the RES, particularly in the spleen. Disproportionate anaemia to parasitaemia indicated destruction of even uninfected RBCs. Role of immune - haemolysis in malaria is disputed. Therefore, oxidant stress and antioxidant defence of erythrocytes in malaria infection was examined. Erythrocyte TBA - reactive material was raised indicating enhanced oxidative damage, despite adequate intraerythrocytic antioxidant integrity consisting of superoxide dismutase, glutathione peroxidase, catalase and reduced glutathione. Detrimental effects of extracellular oxidative stress was significant. Isogroup healthy RBCs when incubated in patients plasma showed increased lactate dehydrogenase and haemoglobin of supernatant indicating haemolysis. This was not so of the incubated patients RBCs in isogroup healthy plasma. Hence extraerythrocytic oxidative factors contribute majority to the anaemia in falciparum malaria.

O - 3.2

LEISHMANIA DONOVANI LIPOPHOSPHOGLYCAN STIMULATES ORNITHINE DECARBOXYLASE ACTIVITY BY PROTEIN KINASE C SIGNAL TRANSDUCTION IN MACROPHAGES

Preeti Kapoor, V Samuel Raj and R Madhubala
School of life Science, Jawaharlal Nehru University, New Delhi-110 067, India.

Leishmania donovani is an obligate intracellular protozoan parasite which resides in macrophages and impairs a number of macrophage functions. *Leishmania* infection is characterized by suppression of cell mediated immunity. In order to become activated to resist or kill an infectious agent, macrophages must be able to respond to external signals by conveying information from the cell surface to the nucleus, a process commonly described as signal transduction. We have undertaken to study the biochemical mechanism whereby *L. donovani* modulates host defences by examining the activity of ornithine decarboxylase (ODC) (EC 4.1.1.17), an early gene, in J774A.1 macrophages

exposed to lipophosphoglycan (LPG). LPG is a glycoconjugate which has been identified as a major surface molecule of the *Leishmania* species. Macrophages stimulated with purified LPG or *L.donovani* promastigotes were found to have elevated ODC levels. The simulation of ODC activity was both dose and time dependent. Maximum stimulation was observed with in 30 minutes of LPG treatment and the activity went down by 3hrs. Pretreatment of macrophages with protein kinase C (PKC) inhibitors staurosporine and phorbol myristic acetate (PMA) for 15 min and 18 hr respectively followed by LPG treatment for 1 hr has shown that LPG causes elevation of ODC phosphatases 1 and 2A and has little effect on protein - tyrosine phosphatases, did not abrogate the effect of staurosporine on LPG stimulated ODC activity. Thus it seems likely that a protein tyrosine kinase is involved in the signal transduction pathway. LPG also impaired the activity of the PKC signal transduction pathway as justified by the use of PKC pathway activators 1-oleoyl-1-acetyl-glycerol (OAG) and calcium ionophore A 23187. PKC was also found to play a vital role in *L. donovani* infection of host macrophages.

O - 3.3

PURIFICATION AND CHARACTERIZATION OF EXCRETORY-SECRETORY PRODUCTS OF *GIARDIA LAMBLIA*

Harpreet Kaur[1], S Ghosh[1], H Sandhu[1], V K Vinayak[2] and N K Ganguly[1]
1. Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India
2. Department of Biotechnology, Govt. of India, New Delhi.

Giardiasis is a cosmopolitan protozoal infection of the intestinal tract. Despite the considerable morbidity caused by giardiasis, little is known about the biology of the parasite as well as the pathophysiology of the diarrhoea associated with the infection. *Giardia lamblia* is known to shed its surface components or release excretory-secretory products (ESP) during *in vitro* cultivation. The exact nature and biological role of ESP in terms of immunobiology and pathophysiology of the parasite was not known. We have purified one ESP of *G. lamblia* by a combination of ammonium sulphate precipitation, affinity chromatography on cyanogen bromide activated Sepharose CL-4B coupled to IgG of crude giardial extract followed by gel filtration chromatography on SW 300 column in the FPLC system. The homogeneity of the purified protein was confirmed in native gel electrophoresis. It revealed a single band of molecular weight 58kDa on SDS-PAGE as also confirmed by gel filtration on Superose 6HR 10/30 column. The purified ESP was a thermolabile glycoprotein and was recognised by the pooled *G. lamblia* positive patient sera which suggested it to be an immunodominant parasite product. The ESP was sensitive to pepsin, trypsin and pronase. It specifically bound to con A, PWM and PHA indicating the presence of α- methyl-D-mannoside, n-acetyl-β-D-glucosamine and n-acetyl-β-D-galactosamine residues in the carbohydrate portion of the protein. The purified ESP caused fluid accumulation in the rabbit ileal loop.

The oral immunisation of mice with purified ESP led to a complete failure of *G. lamblia* colonization following challenge inoculation of these animals with trophozoites. The prior immunisation of mice with ESP resulted in a significant stimulation of local immunity as evidenced by a significant enhancement of T helper/inducer activity along with a significant increase in IgA bearing cells. Further, the presence of anti ES antibodies in the serum of immunised as well as immunised-challenged animals indicated the stimulation of the systemic lymphoid system. This suggests that ESP is highly immunogenic and it could be one of the major antigens of *G. lamblia* responsible for protection from the infection.

O - 3.4

GAMETOCYTOGENESIS IN LOCAL *PLASMODIUM FALCIPARUM* ISOLATES AND CLONES *IN VITRO*

Neera Mehra[1] and V K Bhasin[2]
1. Department of Zoology, University of Delhi, Delhi 110 007.
2. Swami Shardhanand College, University of Delhi, Alipur, Delhi 110 036, India

Plasmodium falciparum isolates collected from a small endemic area of perennial transmission varied in their ability to form gametocytes *in vitro*, an essential determinant in natural transmission of malaria. These isolates, in general, exhibited higher ability to form gametocytes than the isolates studied for gametocytogenesis in non-malarious areas and retained the ability to form gametocytes despite prolonged continuous cultivation. Clonal variations within an isolate were studied with regard to gametocyte production. The clones differed extensively in their ability to produce gametocytes; six of them could not form gametocytes in vitro and rest of them produced gametocytes to variable extent. No gross disparity was observed with respect to time taken from differentiation and maturation of sexual stages in different clone populations. Nine days were required for the formation of morphologically mature micro- and macro-gametocytes *in vitro*. Based on Hawking's classification of different stage of gametocytes the residence time of stage I to V was determined. Genotypic diversity of these isolates and some clones was demonstrated using radiolabelled synthetic 21-oligomer probe, conspicuous restriction fragment length polymorphism was observed in isolates. However, the same probe has been unable to discriminate between phenotypically distinct gametocyte forming and non-gametocyte forming clones.

O - 3.5

STUDIES ON THE SUSCEPTIBILITY OF LABORATORY ANIMALS TO *FASCIOLA GIGANTICA*

P K Bhatnagar[1], D K Gupta[2] and A Prasad[1]
1. Division of Parasitology, Indian Veterinary Research Institute, Izatnagar.
2. Department of Zoology, Bareilly College, Bareilly.

Records on the suitability of laboratory animals as ideal experimental hosts for *Fasciola hepatica* have been proposed from time to time and rats, mice, rabbits and guinea pigs have been recognised as satisfactory models. Similar studies on *F. gigantica* have shown rats and mice to be refractory or rarely susceptible but reports on the susceptibility of rabbits and guinea pigs are at variance and, therefore, the present study was undertaken to evaluate these animals as suitable laboratory models for the aforementioned trematode. During the experimental protocol, 36 rabbits and guinea pigs were divided in six different groups of six animals each, of which group VI of both the animals was treated as control. The metacercarial dose for rabbits (100-400) and guinea pigs (25-150) was administered *per os*. Apart from alopecia in some rabbits, no clinical symptoms were apparent. The necropsy examination of the liver indicated that 70% of rabbits and 66.7% of guinea pigs were affected with immature flukes. Moreover, variations in metacercarial dose had no significant effect on the susceptibility and establishment of the fluke in the liver as the maximum number of flukes recovered was 5, irrespective of the dose. These results have been adequately supported by applying chi square test (χ^2). There was no significant increase in fluke size from 39 days p i onwards. The flukes recovered from rabbits (size 18 mm x 3 mm) were more than double in size as recovered from the liver of guinea pigs.

O - 4.1

EFFICACY OF POUR-ON FLUMETHRIN FORMULATION AGAINST LICE INFESTATION IN BUFFALOES

Satish K Garg, Rajesh Katoch* and Chandra Bhushan*
Department of Pharmacology and Veterinary Parasitology,* C.S. Azad University of Agriculture & Technology,
Mathura Campus, Mathura - 281 001, India.

Flumethrin is known to be an effective acaricide against ticks of cattle and sheep. Present study was planned to evaluate the *in vitro* and *in vivo* efficacy of pour-on formulation of flumethrin (Bayticol, 1% W/V; Bayer, Germany) against buffalo lice (*Haematopinus tuberculatus)*. For *in vitro* studies, 150-200 adult lice were collected from buffalo calves and randomly divided in 6 groups of 25 lice in each to determine the LC_{50} value of Bayticol. Lice populations were counted in 10 x 10 cm. area at each of the 5 sites on the head, neck, shoulders, flank and rump regions of all the 25 animals. Bayticol pour-on (1 mg/kg) was evenly applied along the mid-dorsal line extending from base of the head to the base of the tail of 15 animals while the remaining 10 animals served as untreated controls in which liquid paraffin was applied at the same dose levels. The number of lice were counted on day 3,7,14 and 21 post-application and the results expressed as the per cent reduction in lice population.

LC_{50} value of Bayticol against lice of buffaloes could not be calculated as it was rather baffling to note that buffalo lice could not be maintained under *in vitro* conditions as all the lice died with in a period of 12 hrs. and the mortality was time-dependent. Bayticol application at the recommended dose level (1 mg/kg) resulted in 100 percent reduction in lice population on day 3,7,14 and 21, however, presence of lice could be noticed on day 24. These observations suggest that Bayticol pour-on is cent per cent effective in checking the lice infestation in buffaloes, however, compared to its residual action for 42 days against cattle ticks, the residual action against buffalo lice was found to be less than 24 days.

O - 4.2

EFFECT OF LEVAMISOLE ON THE FUMARATE REDUCTASE SYSTEM OF *M. EXPANSA*

Sharada A and Raghavendra Rao B
Dept. of Zoology, Nizam College, Osmania University, Hyderabad-500 001.

The action of Levamisole on the fumarate reductase in the parasite *M. expansa*, a common intestinal parasite of sheep is studied. The drug Levamisole is known to block the fumarate reductase and succinate oxidation in nematodes. This, being a broad spectrum drug, the study has been undertaken to evaluate its effect on the fumarate reductase

system in the cestodes. The *in vitro* study reveals that Levamisole effectively reduces the enzyme activity in the immature, mature, gravid regions and the total parasite.

O - 4.3

EFFECT OF ALBENDAZOLE ON THE DEAMINASES OF *RATTUS RATTUS* INFECTED WITH *H. DIMINUTA*.

Suhasini R and Raghavendra Rao B
Department of Zoology, Nizam College, Osmania University, Hyderabad - 500001.

The deaminases specially Adenosine deaminase (ADA) is associated with purine pathway converting adenosine to inosine. This purine metabolism is an important process particularly for the immune system, which has gained importance of late. The absence of ADA ceases the maturation of lymphocyes B & T cell due to which they become sparse & dysfunctional, causing severe immunodeficiency diseases. A study was therefore, undertaken on the rats infected with *H.diminuta* which were administered with albendazole (ABZ), the levels of deaminases (ADA & AMO) were estimated in tissues like kidney, liver, intestine and spleen. The results indicated a significant decrease in all these tissues. This indicates that the prolong usage of ABZ can lead to immunological changes.

O - 4.4

EFFICACY OF LEVAMISOLE HCL (HELMONIL-150) AGAINST STRONGYLOSIS IN BUFFALOES

Md Hafeez and C Sridevi
Department of Parasitology, College of Veterinary Science, Acharya N.G. Ranga Agricultural University, Tirupati-517 502 (A.P.), India

In a clinical trial conducted to test the efficacy of levamisole HCL (Helmonil-150), against strongylosis in naturally infected buffaloes it was observed that, buffaloes suffering from strongylosis after treatment with Helmonil-150 tab. at the rate of 7.5 mg/kg body weight showed a reduction of 80.5, 88.8 and 96.8 per cent on 2nd, 3rd and 4th day respectively after treatment. A cent per cent reduction was noticed on 7th day after treatment. Most of the symptoms exhibited, before the treatment, in this treated group of animals were alleviated on 3rd day and the condition of treated animals was restored to normalcy by 4th day after treatment.

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O - 4.5

A NOVEL APPROACH TO THE TREATMENT OF ACUTE BLOOD-STAGE MALARIA : COMBINATION OF LOW DOSE CHLOROQUINE AND IL-12

Karkada Mohan and Mary M Stevenson
Montreal General Hospital Research Institute, McGill University, Montreal, PQ, Canada

Development of resistance by *P. falciparum* to many anti-malarials coupled with resurgence of disease in many developing countries make it essential to develop new drugs or to modify current therapeutic strategies against this potentially fatal infection. Encouraged by our previous observation that 0.1 µg IL-12 treatment for 6 days results in up to 100% survival of *P. chabaudi* AS susceptiible A/J mice, we examined the protective efficacy of combining low doses of chloroquine (CQ) and IL-12 to treat blood-stage infection. While untreated mice developed parasitemias > 50% and succumbed to infection, mice receiving half the therapeutic dose of CQ (total 12.5 mg/kg) had a peak parasitemia of about 20% and survived infection. Combining this dose of CQ with half the protective dose of IL-12 (total : 0.3 µg/mouse) resulted in a significnat reduction in parasitemia of 3%, a longer prepatent period, a delayed peak parasitemia and a survival rate of 100%. Preliminary findings also suggest that by reducing the dose of IL-12 further to 0.2 µg/mouse and starting treatment after the prepatent period, on day 3, the protective efficacy of this IL-12+CQ combination essentially remains the same as observed above. Moreover, mice receiving this therapy showed significantly higher hematocrits, red cell counts and percentage reticulocytes compared to untreated mice. Thsi combined therapy may have several advantages : i) low dose CQ provides the opportunity to increase dosage if required, thereby prolonging the development of drug resistance; ii) a low protective dose of IL-12 may allow clinical use at non-toxic levels; iii) since Il-12 treatment can be started along with CQ after detection of patent parasitemia, it may have applicability to clinical use; iv) since IL-12 can boost erythropoiesis during malaria, this therapy may prevent life threatening anemia associated with severe *P. falciparum* malaria; and v) as IL-12 induces a protective Th1 type host response against malaria parasites, following this combined therapy, the level of immunity developed might be relatively long lasting and protective against re-infection. *(Supported by NIAID, NIH and MRC Canada).*

O - 4.6

J Singh
(Abstract not received)

O - 4.7

ENHANCEMENT OF MEMBRANE DAMAGE BY SAPONINS ISOLATED FROM *ACACIA AURICULIFORMIS*

Santi P Sinha Babu, Debapriya Sarkar, Naba K Ghosh, Arpita Saha, Nirmal C Sukul and Sh Bhattacharya
Department of Zoology, Visva-Bharati University, Santiniketan - 731 235, West Bengal, India

Acaciaside A and B, two acylated triterpenoid bisglycosides isolated from the funicles of *Acacia auriculiformis,*are known to have antihelmintic activity. Since the saponins contain a conjugated unsaturated system which is highly susceptible to peroxidation, we investigated the interaction of saponins and membrane using rat liver microsomes as our model. Microsomal membranes were incubated with saponins at 30°C and 2 h. Following incubation, lipid peroxidation was measured in terms of malondialdehyde (MDA) and Conjugated Diene (CD). Our results showed that incubation of microsomal membranes with saponins increased both MDA and CD in a concentration-dependent manner. The results suggest that, in our model, saponins enhance the membrane lipid peroxidation. We suggest that the conjugated unsaturated system is involved in producing the damaging effects of saponins to the parasite membrane through peroxidation.

O - 4.8

COMBINED ACTION OF INHIBITORS OF S-ADENOSYLMETHIONINE DECARBOXYLASE WITH AN ANTIMALARIAL DRUG CHLOROQUINE ON *PLASMODIUM FALCIPARUM*

Bhagwan Das, pashmi Gupta and Rentala Madhubala
School of Life Scienc, Jawaharlal Nehru University, New Delhi - 110 067, India

Methylglyoxal bis (guanylhydrazone), (MGBG) a potent competitive inhibitor of S-adenosyl-L-methionine decarboxylase activity. Berenil, a trypanocidal agent and chloroquine, the commonly used antimalarial resulted in a dose dependent inhibition of *P. falciparum* in vitro. The IC_{50} value of MGBG, Berenil and chloroquine were 224 µM, 40 µM and 42 nM respectively. Parasites treated with different concentrations of MGBG or Berenil were arrested at the trophozoite stage of the erythrocytic cycle. The combined action of chloroquine (10 nM) with either Berenil (0.1 mM) or MGBG (0.1 mM) on *P. falciparum* growth showed an additive inhibitory effect. The effect of these inhibitors alone and in combination on polyamine biosynthesis is also reported.

O - 4.9

MALARIA PIGMENT AND MOLECULAR MECHANISM OF ACTION OF BLOOD SCHIZONTOCIDAL ANTIMALARIALS

Amit Vikram Pandey and Babu Lal Tekwani

Division of Biochemistry, Central Drug Research Institute, Lucknow.

During the intraerythrocytic proliferation malaria parasite degrades large amount of hemoglobin for its nutritional requirements. Toxic free heme is released as a by-product of this reaction. Malaria parasite detoxifies heme by converting it into malaria pigment known as hemozoin. Hemozoin is a polymer of heme units linked through an iron carboxylate bond. Pigment remains inert inside the parasite and sequestered to various tissues of the infected host after rupture of parasitized erythrocytes. Blood schizontocidal antimalarials inhibit the polymerization of heme thus blocking the heme detoxification pathway of the parasite. We have studied the interaction of hemozoin with blood schizontocidal antimalarials. All these antimalarials have the tendency of binding with heme. We have found that different blood schizontocidal antimalarials inhibit the polymerization of heme and interact with hemozoin to convert it back into monomeric heme units. Further, mechanism of hemozoin formation and its inhibition by chloroquine has been studied using synthetic peptides which simulate the heme binding sites of the malarial histidine-rich protein (HRP) which is responsible for the heme polymerization. These studies identify malaria pigment as a potential target for development of antimalarial drugs. Data from the heme interaction studies indicate the mechanism of drug accumulation and action, which offers a way to counter the problem of drug resistance that results due to sublethal drug accumulation. Efficient heme binding drugs are expected to negate this process yielding better antimalarials.

O - 4.10

TREMATOCIDAL ACTIVITY OF SOME TRADITIONAL MEDICINAL PLANTS IN NORTH - EAST INDIA

B Roy[1] and V Tandon[2]
1. Department of Zoology, North-Eastern Hill University, Shillong 793 022, India.
2. Pachhunga University College, Aizawl (Mizoram), India.

Trematodicidal efficacy of some indigenous plants consumed by the native tribals in Meghalaya and Tripura (North-East India) was tested. *In vitro* exposure at 37°C ± 1°C of the amphistomid parasite, *Orthocoelium* sp. (from rumen of cattle) to the alcoholic extract of *Alpinia allugaris* (shoot), *Cannabis Sativa* (leaf) and *Flemingia vestita* (root-tuber peel) at a concentration of 10 mg / ml of phosphate buffered saline (PBS) caused paralysis and death of the flukes within 0.6 - 0.8, 0.5 - 0.6 and 0.5 - 0.7 hr respectively. The commercial flukicide Oxyclozanide at the same concentration led flukes to paralysis and death within 1.5 - 2.0 hr. In comparison, the control parasites incubated in PBS alone survived for 46 hr. Scanning electron microscopic observations revealed deformity and disruption of tegument and sensory papillae in the treated flukes. The present *in vitro* studies indicate that the crude extract of all the three plants may have trematodicidal properties which need to be further investigated.

O - 4.11

MOLECULAR ANALYSES OF DRUG-RESISTANCE IN GAMBIAN ISOLATES OF *PLASMODIUM FALCIPARUM*

Manoj T Duraisingh, Peter Jones, Idrissa Sambou, Lorenz von Seidlein, Margaret Pinder and David C Warhurst
London School of Hygiene and Tropical Medicine, Keppel Street, London, WCIE 7HT
Medical Research Council Laboratories, Fajara, The Gambis

Resistance in *P. falciparum* is increasing throughout the world seriously compromising treatment, yet the molecular basis of resistance to chloroquine (CQ) and the other quinoline antimalarials remains uncertain. In The Gambia resistance may involve mutations in the *pfmdr*1 gene (asn86 to try-86), a plasmodial homologue og the human multidrug transporter. We conducted *in vitro* drug-tests on 28 isolates obtained from patients with uncomplicated malaria at the MRC Laboratories, Fajara, The Gambia, with the standard antimalarials CQ, mefloquine (MEF), halofantrine (HAL), quinine (QUIN), and with artemisinin (QHS) and dihydroatemisinin (DHA). Polymorphism at the 86- and the 184- codons of *pfmdr*1 in these isolates was typed by PCR followed by restriction digestion. Our results indicate that the try-86 codon is associated with increased sensitivity to MEF and HAL, in contrast to the earlier observation of increased resistance to CQ. The isolates with the try-86 mutation also had an increased sensitivity to the artemisinins. We are currently analysing these isolates for mRNA expression and gene copy number of *pfindr*1 to determine whether increased expression contributes to sensitivity. The results of these analyses will be presented.

O - 4.12

ANTIMALARIAL EFFICACY OF ALBENDAZOLE

Geoffrey S Dow, Amand J Ohara, James A Reynoldson and R C Andrew Thompson
School of Veterinary Studies, Murdoch University, Murdoch, Western Australia 6150, Australia.

Albendazole is a benzimidazole anthelmintic widely used for treatment of a variety of parasitic infections of humans and is thought to be active against *Plasmodium falciparum in vitro*. Preliminary experiments have also found it to have activity against the rodent

malaria parasite, *Plasmodium berghei, in vivo.* In this study we conducted a more thorough investigation; treating *P. berghei*-infected rats with a range of albendazole doses, and monitoring parasitaemia to determine efficacy. The SD_{50} (dose that suppressed parasitaemia by 50%) and SD_{90} values of the compound were approximately 30mg/kg/day and 60mg/kg/day respectively for three days. However, albendazole treatment exacerbated many of the adverse effects of *Plasmodium* infection (eg anemia). In other experiments we assessed the *in vitro* efficacy of albendazole and its sulfoxide and sulfone metabolites against *P. berghei* using the tritiated hypoxanthine incorporation assay. These results will be discussed in the light of the *in vivo* efficacy and toxicity of the compound, and the efficacy of conventional antimalarial drugs in similar test systems.

O - 4.13

ANTHELMINTIC EFFICACY OF THE AQUEOUS EXTRACT OF ASAFOETIDA IN COMMON POULTRY WORM *ASCARIDIA GALLI.*

Shalini Nagich and Kavindra Singh
Pest and Parasite Research Laboratory, Post graduate Department of Zoology, Bareilly College P.O. Box 15, Bareilly - 243 005.

The resinous root exudate of *Ferula assafoetida* (heing) which is commonly used as spice was purchased from the local market and tested for its anthelmintic efficacy *in Vitro* against common poultry worm *Ascaridia galli.* A suspension of this gum like substance was prepared by mixing lgm of assafoetida in 20ml of double distilled water. Different concentrations used were obtained by diluting this stock solution. Under laboratory condition (Temp. 35°c), the suspension proved to be 100% effective at concentrations of 4%, 3% and 2%, as it caused complete mortality (indicated by loss of motility) of worms after a period of 5, 7 and 9 hours respectively, when exposed *in vitro.* 1% suspension however caused mortality of 80% worms after an exposure of 11 hours. The effect of the suspension was also examined on Glucose uptake, the activity of non specific phosphomonoesterases and lactic acid production of the parasites to find out its possible mode of action. It caused an increase in glucose uptake, lactic acid production and acid and alkaline phosphomonoestrase activity.

O - 4.14

CLINICAL TRIAL WITH &-B-ARTE-ETHER IN UNCOMPLICATED *PLASMODIUM FALCIPARUM* CASES IN MADHYA PRADESH

Neeru Singh,[1] M M Shukla,[1] Mukesh Khapra,[2] O P Asthana,[3] and A K Gupta[4]
1. Malaria Research Centre, Field Station, Government Medical College Building, Jabalpur - 482 003.
2. Department of Medicine, Government Medical College, Jabalpur - 482 003.
3. Department of Experimental and clinal Medicine, Central Drug Research Institute, Lucknow - 226 001.
4. Parhology Laboratory, Military Hospital, Jabalpur - 482 001.

Clinical trial (phase III) was carried out by Malaria Research Centre, Jabalpur in collaboration with department of Medicine, Govt. Medical college, Jabalpur. In all, 32 normal healthy patients with uncomplicated *Plasmodium falciparum* were selected for the study with their consent. They were admitted in hospital for 4-7 days and were given 3 injections of &-B arte-ether (150 mg) once a day intramuscularly on 3 consecutive days. Further weekly followup of these patients was done by visiting their houses.

At the time of hospitalization, all the patients were presented with fever with chills and rigor. The fever in 44% patients got controlled within 24 hrs and 53% patients became afebrile within 72 hrs without administration of antipyretic drugs. Only one patient remained febrile after administration of 3 knjections.

The asexual parasitaemia was cleared from peripharal blood smears within 24 hrs in 62.5% patients and remaining patients became aparasitamic within 48 hrs. Only gametocytes were seen in 37% patients upto 7 days. Follow up results revealed that blood smear was found positive again for asexual parasites within 28 days in 2 patients (6%). No adverse effects were observed clinically of parasitologically.

O - 4.15

IN VITRO SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* ISOLATES TO DHFR INHIBITORS AND OTHER ANTIMALARIALS

Lathika Nair and V K Bhasin
Department of Zoology, University of Delhi, Delhi 110 007.

Proguanil and pyrimethamine exhibit their antimalarial virtues by preferentially inhibiting the dihydrofolate reductase (DHFR) enzyme of parasites. Pyrimethamine has been deployed on a much larger scale than proguanil. These drugs have been extremely useful in treatment of chloroquine resistant falciparum infection but the limiting factor has been the quick development of resistance among the parasite populations against these drugs. Pyrimethamine resistance is known to be widely distributed in all malaria endemic areas of the globe, including India. Proguanil has not been used in India for over fifty years now. It should, therefore, be of interest to know the susceptibility of pyrimethamine resistant and sensitive isolates of Indian origin to cycloguanil (the active metabolite of proguanil). These considerations prompted us to perform a series of experiments with a view to assess the potential usefulness of cycloguanil in circumventing and combating the problem of pyrimethamine resistance in falciparum. Sensitivity of a few local isolates and clones derived from them to these drugs was determined *in vitro*. All the isolates were found to be sensitive to cycloguanil. Data on cross resistance, of pyrimethamine sensitive and resistant lines, to cycloguanil, methotrexate and other groups of antimalarial drugs will be presented. There has been no synergistic activity observed between cycloguanil and pyrimethamine.

O - 5.1

CEREBRAL MALARIA IN INDIAN ADULTS :

Dhanpat Kochar
Cerebral Malaria Clinic, Section of Neurology, Department of Medicine, S.P. Medical College, Bikaner.

India has witnessed an alarming increase in *P.falciparum* malaria in 1990s. The reflection is parallel in Rajasthan and it accounts for 40% share in comparison to 15% in 1976. We had also observed 2 large scale epidemics in 1992 and 1994. The probable cause of breaks is change in ecosystem, increase in annual rainfall, cultivation of shrubs around water logging area near canal and adaptation of vector to local geographical conditions along with the problem of resistance of parasite and vector to the commonly used antimalarial drugs and insecticides respectively. Severe infection in the form of cerebral malaria is associated with high mortality and morbidity, specially in non-immune population. We studied prospectively 415 adult patients of strictly defined cases of cerebral malaria admitted in medical college hospital during 1992-1996. All patients were treated by I.V./oral quinine and specific syndromes were managed according to WHO guidelines. 138 (34.43%) patients expired and mortality was highest in pregnant ladies. Apart from fever and unconsciousness in all the patients, other features included convulsions (20.75%), neck rigidity (19%), psychosis (5.41%), cerebellar ataxia(3.97%), aphasia (2.78%), conjugate deviation of eyes (2.02%), hemiplegia (1.80%) and extrapyramidal rigidity (1.08%). The important neurological sequelae in survivors at the end of second week of hospitalization were cerebellar ataxia, hemiplegia, psychosis, extrapyramidal rigidity, isolated 6th nerve palsy and peripheral neuropathy, all of which recovered completely in further follow-up. We also encountered 12 cases of delayed cerebellar ataxia (3 in 1992, 7 in 1994 and 2 in 1995), which is a self limiting immunological syndrome and has also been reported from Sri Lanka. Coexisting other pernicious syndrome were in the form of severe anaemia (6.07%), jaundice (5.82%), renal failure (5.32%), algid malaria (5.06%), pulmonary oedema (4.56%) and haemoglobinuria (14.09%). Very high mortality was observed in patients having 3 or more pernicious syndromes together. Based on our observation of increase in mortality and morbidity in patients having convulsions in cerebral malaria. Phenobarbitone significantly reduced ($p<0.001$) the incidence of subsequent convulsions from 23% in those who did not receive phenobarbitone to 2.9% in those who received prophylactic I.M. phenobarbitone. A detailed ophthalmoscopic examination of 214 patients of cerebral malaria revealed that retinal haemorrhages, retinal oedema and disc pallor were associated with poor outcome. Statistically, these changes are just significant ($p<0.5$), however papilloedema and blurred margins of disc were not associated with increased mortality. The high mortality in pregnant ladies, should be emphasized, as early and prompt treatment can be rewarding. Knowledge of self limiting course of neurological sequelae may be helpful in reducing economic strain of expensive investigations and treatment.

O - 5.2

IMMUNODOT ENZYME ASSAY OF PLASMODIAL LACTATE DEHYDROGENASE FOR DIAGNOSIS OF MALARIA

Deep C Kaushal and Nuzhat A Kaushal
Central Drug Research Institute, Lucknow - 226 001.

Malaria continues to be a major health problem occupying the top position among the parasitic diseases. Improved diagnostic methods are being sought for effective treatment and eventual eradication of malaria. The conventional microscopy is time consuming, requires certain amount of expertise and thus not very ideal for situations where mass testing is needed. This emphasises the need to develop alternative diagnostic methods for effective management of malaria. Our earlier studies have demonstrated that the lactate dehydrogenase (LDH) of malaria parasites is immulogically distinct from the host enzyme. The polyclonal antibodies, produced against the affinity purified *Plasmodium knowlesi* LDH showed specificity to plasmodial LDH and are directed against the epitope(s) other than the catalyic site. In the present study, these anti-LDH polyclonal antibodies were used to develop an immunodiagnostic test (immunodot enzyme assay of plasmodial LDH) based on detection of plasmodial LDH in patient blood sample. The test involves the histochemical staining of the parasite LDH after capturing by the plasmodium specific anti -LDH antibodies immobilised on nitrocellulose paper strips. The immunodot enzyme assay of plasmodial LDH was evaluated using blood samples from malaria and other infections. Out of 317 microscopically positive malaria blood samples, 314 blood samples showed positive immunodot assay of LDH while all the 145 microscopically negative cases were found negative by our test. The blood samples from other infection and non-endemic controls were found negative by immunodot enzyme assay of LDH. The test is simple to perform, read visually, highly specific with a sensitivity of ~99% and thus suitable for rapid and accurate diagnosis of malaria.

O - 5.3

CLONING AND EXPRESSION OF A FILARIAL EXCRETORY-SECRETORY ANTIGEN

Nuzhat A Kaushal, Deep C Kaushal, Ronald E Blanton and James W Kazura
CDRI, Lucknow, India.
CWRU, Cleveland, Ohio, USA.

Filariasis is a major public health problem in tropical and sub-tropical regions of the world. Effective chemotherapeutic and immunological remedies against filariasis are lacking and definitive diagnosis of the disease is still a problem. Due to the relative insensitivity of parasitological diagnosis, immunological methods have been used for diagnosing filarial infections. However, these methods require large amounts of scarce parasite material. An approach to obtain these antigens in large quantities is by

recombonant DNA technology. Our earlier studies have demonstrated certain filarial antigens of diagnostic significance and in the present study cloning and expression of these antigens was done. Immunoscreening of a X gtll cDNA expression library of *Brugia malaye* (human filarial parasite), using polyclonal antibodies against the filarial excretory-secretory antigens (detecting filarial circulating antigen), lead to the identification of 3 cDNA clones (Bm1-1,Bm3-2, Bm5-1). The DNA from these clones were PCR amplified, inserts were characterised and subcloning was done in pRSET expression vector. Out of the 3 clones, only Bm5-1 pRSET plasmid showed significant exprerssion of protein. The recombinant protein was purified and the immuoreactivity was tested in ELISA using sera from filarial and non-fialrial patients. The recombinant protein was found to be highly immunoreactive as shown by high reactivity with antibodies in filarial patients sera. No significant reactivity was observed with non-filarial patients sera, thereby suggesting the filaria-specificity of the recombinant protein.

O - 5.4

MULTIMODALITY EVOKED POTENTIALS IN CEREBRAL MALARIA- A PROGNOSTIC SIGNIFICANCE

Banshi Lal Kumawat, Dhanpat Kochar, Mahendra Halwai and Sanjay Kochar
Department of Medicine, S.P. Medical College, Bikaner, Rajasthan, India

Multimodality evoked potentials (brainstem auditory evoked potentials; BEAPs & right median nerve somatosensory evoked potentials; SSEPs) were performed in 25 adult patients of cerebral malaria in acute stage. Glasgow coma scale was used to assess the level of consciousness. The abnormalities of BAEPs were delayed peak latency of wave III in 13/25 (52%) & wave V in 20/25 (80%) patients & delayed interpeak latencies (IPLs) of wave I-III in 9/25 (36%), wave I-V in 15/25 (60%) & wave III-V in 12/25 (48%) patients. In SSEPs delayed N_{20} was seen in 11/25 (44%); delayed IPLs of N_{13} - N_{20} (central conduction time; CCT) in 12/25 (48%) patients. Distorted N_{20} was recorded in 12/25 (48%) patients Both N_{13} - N_{20} IPLs in SSEPs and wave III-V IPLs in BAEPs were delayed in 5 patients and all of them expired. Delayed N_{13} - N_{20} with normal III-V IPLs was present in 7 patients and 2 of them died, whereas delayed III-V IPLs with normal N_{13} - N_{20} was present in 7 patients, and one of them expired. In remaining six patients both the parameters were normal and one of them died. So, it was observed that BAEPs/SSEPs alone was not useful for predicting the outcome of come, whereas abnormalities in both is determinant of worst prognosis. The changes in evoked potentials (BEAPs & SSEPs) could be due to interruption of conduction in central pathways because of structural changes due to patechial hemorrhages and malaria granuloma at multiple levels in the brain including brainstem.

O - 5.5

CONVULSIONS IN CEREBRAL MALARIA, ITS EFFECT ON MORBIDITY AND MORTALITY & SUCCESSFUL PREVENTION BY PROPHYLACTIC INTRAMUSCULAR PHENOBARBITONE.

Indu Thanvi, D K Kochar, B Kumawat, N Agarwal, A Joshi.
Cerebral Malaria Clinic, Dept. of Medicine, Division of Neurology, S.P. Medical College, Bikaner, (Rajasthan) India.

There are various studies showing convulsions as a prognostic indicator in children and adult patients of cerebral malaria. We describe a study on 185 patients of adult cerebral malaria admitted in P.B.M. Hospital attached to S.P. Medical College, Bikaner Rajasthan (India) between August and November 1994. Diagnosis of cerebral malaria was done according to WHO definition and demonstration of asexual form of *P. Falciparum* in peripheral blood film. Detailed neurological examination was done in all the patients at the time of admission, immediately after recovery from comma and at the time of discharge on 15th day. 40 patients (21.62%) had convulsions and 17 (42.5%) of them died during illness, as compared to 45 out of remaining 145 (31.03%) patients without convulsions. The details of neurological sequelae in survivors just after recovery from coma and at the time of discharge are given in table. Severe hypoglycaemia (blood sugar level < 2.2 mmol/l was present in 8 (4.32%) patients. Convulsions were present in 3 of these patients and all the 3 expired in spite of correction of hypoglycaemia.

Cerebral malaria	Total cases	Mortality	Neurological sequelae in survivors	
			After recovery from Coma	On 15th day
With convulsions at the time of admission	40	17 (42.50%)	9 (22.50%)	5 (12.50%)
without convulsions	145	45 (31.3%)	29 (20.00%)	8 (5.55%)

Prophylactic phenobarbitone in patients of cerebral malaria with convulsions (10 mg/kg body wt. I.M. at the time of admission) has shown to decrease the incidence of convulsions in further course of illness. The incidence of convulsions in phenobarbitone treated patients was 3 out of 102 (2.9%) as compared to 19 out of 83 (23%) in those who did not receive phenobarbitone ($p<0.001$). Phenobarbitone also significantly reduced the further occurrence of convulsions in patients who were having convulsions before admission ($p<0.001$).

O - 5.6

OPHTALMOSCOPIC ABNORMALITIES IN PATIENTS OF CEREBRAL MALARIA

Shubhakaran, D K Kochar, B L Kumawat, A Kochar, S P Vyas
Department of Medicine & Ophthalmology, S.P. Medical College, Bikaner, (Rajasthan) India.

The role of ophthalmoscopic abnormalities with ultimate prognosis in cerebral malaria is unclear. Retinal hemorrhages have been shown to be a bad prognostic indicator by Looareesuwan et al in Thailand in adults and Kaymbee et al in African children. Davis in Papua New Guinea and Patrick Haslett in Zambian children had observed retinopathy to be of no significance. Papilloedema and extra macular oedema were observed to be of significance by Lewallen et al in Malawian children where as Dhamija and Padhiary had observed papilloedema to be of no significance in Indian adults. No large study is available and the importance of fundus findings in relation to prognosis is still not clear. In our study, 214 adult patients of cerebral malaria were examined by direct and if required, by indirect ophthalmoscope between 1994-1996. The important findings observed were in the form of retinal haemorrhage in 20 (9.34%) patients papilloedema in 14 (6.54%), blurring of disc margins in 16 (7.47%), retinal oedema in 4 (1.86%), disc pallor in 4 (1.86%), and vitreous haemorrhage in 1 (0.46%). Other findings were hyperaemia with blurred margins of disc in 5 (2.33%) patients, retinal haemorrhage with papilloedema in 3 (1.40%), hyperaemia with blurred margins of disc with retinal oedema in 1 (0.46%). The important findings associated with increased mortality were, retinal haemorrhage [5 out of 20 (25%)] and retinal oedema [1 out of 4 (25%)], disc pallor was observed in 4 patients and all of them expired. The patient with vitreous haemorrhage initially presented with blindness before developing unconscioness and expired after 3 days of standard antimalaria treatment.

Ophthalmoscopic abnormalities in the form of retinal haemorrhage, retinal oedema and disc pallor are associated with poor outcome as compared to the patients without these findings. These changes are just significant ($p<0.5$) statistically so absolute statement cannot be made. Papilloedema (21.42%) and blurred disc margins (18.75%) were not associated with increased mortality as compared to the patient with normal fundus (20.56%). All other findings were not associated with mortality. Vitreous haemorrhage can also be a cause of blindness in patients of falciparum malaria besides cortical blindness, retrobulbar neuritis and chloroquine induced transient blindness. The details of these results will be discussed.

O - 5.7

COMPARATIVE STUDY OF ANTIGEN DETECTION (ELISA METHOD) AND MICROSCOPICAL EXAMINATION FOR *PLASMODIUM FALCIPARUM*

Nagat A Muqbil[1], S Sharma[1] and B Lal Gupta[2]
1. Department of Zoology, University of Rajasthan, Jaipur, India
2. Dr. B. Lal's Clinical Laboratory, B-3, New Colony, Panch Batti, Jaipur, India

77 human blood samples of high grade fever cases with rigors were collected from different localities in and around Jaipur (India), and then investigated for *P. falciparum* infection by antigen detection (ELISA method) and conventional microscopic Examination (P.B.F. method). These samples were collected randomly during the period from August 1996 to February 1997. 17 samples (22%) were found positive for *P. falciparum* by ELISA tesantigen detection while only three (3.8%) by the Conventional Microscopic Examination (P.B.F.), suggesting ELISA is more superior test in detection of *P.falciparum.* Our study also revealed that Elisa can not replace P.B.F. method completely, more particularly, in cases shown negative by ELISA, but symptomatically resembling malaria. Such cases have to be reaffirmed for the absence of malaria infection by P.B.F. method also.

O - 5.8

SERODIAGNOSIS OF INDIAN VISCERAL LEISHMANIAS WITH A RECOMBINANT ANTIGEN FROM THE OrfF LOCUS OF LD1.

V Samuel Raj, Anirban Ghosh, Kenneth Stuart and R. Madhubala
School of Life Sciences, Jawaharlal Nehru University, New Delhi and 'Seattle Biomedical Research Institute, Seattle, USA

Visceral Leishmaniasis (VL) is a widespread and often fatal disease caused by *Leishmania donovani.* In India, Kala-azar is a major public health problem particularly in parts of Bihar and West Bengal. Diagnosis of VL relies mostly on the demonstration of parasites in bone marrow or spleen biopsy tissue. Development of less invasive means of diagnosis is the need of the hour. The various diagnostic methods include the application of enzyme-linked immunosorbent assay (ELISA) to identify relevant antigens with patients sera or to detect Leishmania specific antibodies in patient's sera using whole cell lysates. We have cloned orfF of LD1 locus in a bacterial expression vector pET-17b. The gene encodes a protein of ~34 KD. The universal feature of orfF is: it is amplified in several natural isolates of *L.donavani*hence representing anmportant gene of the parasite. The purified orfF recombinant antigen is used to check its immunodiagnostic potential by enzymelinked immunosorbent assay with sera from a total of 78 symptomatic splenomegalic cases from endemic area of Bihar. All sera samples from healthy individuals and patients were first evaluated by using the direct agglutination test. Out of 49 clinically confirmed cases 94% were found to be orfF positive when ELISA was performed using 5ng of recombinant orfF antigen where as 71% of the above samples

showed positivity when tested with 500ng of soluble antigens. Endpoint titre of 10 VL cases using 5 ng of orfF fusion protein was 1:1280 where as the reciprocal endpoint titre with endemic, nonendemic normals, malarial, tuberculosis and filarial cases was >20. Ten Cutaneous Leishmaniansis cases from Turkey when assayed for orfF recombinant antigen gave reactions ranging from negative to marginally positive.

O - 5.9

A Ghosh
(Abstract not received)

O - 5.10

PLASMODIUM FALCIPARUM : DETECTION AND STRAIN IDENTIFICATION BY POLYMERASE CHAIN REACTION.

Amar Bir Singh Sidhu, Lakshmi Natrajan and R. Madhubala
School of Life Sciences, Jawaharlal Nehru University New Delhi-110067

Malaria, a parasitic disease caused by one of the four species of *Plasmodium* is a major cause of morbidity and mortality in many tropical and subtropical countries. Approximately 200 million people are affected each year. The major problem in recent years has been the development of various new strains within all the preexisting *Plasmodium* species. Hence the development of new diagnostic methods for identifying low levels of infection and identification of different strains is of the utmost importance. Earlier studies have shown that prevalence of parasites in human population is greatly underestimated by microscopical examination. So there is an urgent need to look for other methods for diagnosis. This work describes PCR based assay which provides a sensitive specific and reproducible method by which various strains of *P. falciparum* can be identified.

Genes that encode cell surface antigens are generally used to study the variation in different strains of *Plasmodium*. These genes have conserved 5' and 3' ends but contain a region with blocks of repeated sequence which vary in size and DNA sequences from strain to strain. We selected highly conserved regions and chose primers that spanned the repeats in four surface antigens: RESA, MSP-1, MSP-2 and CSP. Giemsa stained thin smears and blood of *P. falciparum* infected patients were used from parasite DNA extraction PCR was performed by using four different set of highly specific primers for surface antigens. Results showed heterogenity in *P. falciparum* population and one strain which was reported as *P.falciparum*infection by the hospital was actually found to have mixed infection. PRC was also done with *P.vivax* and *P.malariae* DNA from different blood samples. Although these primers are specific for *P. falciparum* surface antigens yet they recognized few *P.vivax* and *P. malariae* infected blood samples. These result suggest the applicability of this type of genotyping of epidemiology, monitoring response to therapy, control of drug resistant strains, identifying strains for laboratory and

comparison of geographical distributions of different strains at different time.

O - 5.11

SERODIAGNOSIS OF BABESIOSIS IN BUFFALO

A K Tewari, A K Mishra and J R Rao
Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, U.P., India.

Information on the prevalence of babesiosis, a tick borne, intraerythrocytic protozoan disease in buffaloes, is scanty. India is having the highest number of buffalo population in the world and its contribution to milk, meat, skin and other by-products is significant in the national economy. In the present study, attempt was made for serodiagnosis of babesiosis in buffalo using ELISA and IFAT. Non-availability of anti-bubaline commercial conjugates necessitated their preparation. Anti-buffalo rabbit IgG was raised and two batches were conjugated with horse-radish peroxidase (HRPO) and fluorescein isothiocynate (FITC) separately following standard protocol and used in the tests. A total number of 650 buffalo sera collected from local abattoir were tested. A total of 42 serum samples were found to be positive (6.5%) by ELISA. First batch of 200 serum samples were subjected to IFAT also which detected antibabesial antibodies in 6% samples. It was also interesting to observe that all the serum samples positive to *Babesia bigemina* cross reacted to *Babesia bovis* in ELISA.

O - 5.12

DEVELOPMENT OF MONOCLONAL ANTIBODIES TO *ENTAMOEBA HISTOLYTICA* SPECIFIC COPROANTIGEN AND THEIR POTENTIAL TO DIAGNOSE THE CURRENT AMOEBIC INFECTION

Karnail Singh,[1] N K Ganguly,[1] V K Vinayak[2]
1. Department of Exp. Med. and Biotech, PGIMER, Chandigarh, India.
2. Department of Biotechnology, Govt. of India, New Delhi, India.

Amoebiasis, the enteroinvasive disease caused by the enteric protozoan parasite *Entamoeba histolytica* is a major public health problem in many parts of the world. Conventional methods of diagnosing intestinal amoebiasis based on the parasitoscopy and demonstration of the antiamoebic antibodies have their own limitations. A test system based on the demonstration of the parasite product in the clinical specimens holds considerable promise. In the present study, *E. histolytica* specific coproantigens were identified and purified by affinity chromatography. Antibodies were raised against these amoebic coproantigens and were checked for their specificity. Monoclonal antibodies directed against one of these amoebic coproantigens were raised and characterised. This amoebic coproantigen was physio-chemically characterised. Using the monoclonal antibody, a PoAb - MoAb based sandwich ELISA system was developed and evaluated on the stool samples from the confirmed cases of the intestinal amoebiasis, cases with non-amoebic parasitosis and apparently healthy controls. The test system developed was

found to be 89% sensitive and 100% specific. The results of this study will be discussed in detail.

O - 5.13

ANOPHELES DIRUS TRANSMITTED MALARIA IN A FOREST FRINGED VILLAGE OF ASSAM, INDIA.

Anil Prakash, D R Bhattacharyya, P K Mohapatra and J. Mahanta
Regional Medical Research Centre, N.E. Region (ICMR), Post Box No. 105, Dibrugarh-786 001, Assam, India.

Longitudinal data on ecology and bionomics of *Anopheles dirus* and malaria transmitted by it in an isolated forest fringed village of Assam were recorded at monthly interval between August 1995 and July 1996. Extended malaria transmission was seasonal (May-December), intense closely following the abundance pattern of *An. dirus,* the lone vector in the study area. The cases were uniformly distributed in the village without any clustering. Data suggested that 65% villagers suffered at least once, 23% twice, 7% thrice, 4% four times and 1% more than four times from fever during the study with slide positivity rate (SPR) of 47% and predominance of *P.falciparum* infection (83%). Prevalence of *P.vivax* was high during May to July (ranging 26.8% to 36.4%) and low between August to February (0 to 19%). Malaria prevalence in the community was inversely related to age and significantly higher ($p < 0.05$) in females (SPR 53.5%) than males (SPR 39.2%). Vector density was rain dependent and a good positive correlation ($r=0.721$) was found between the density of *An.dirus* and the amount of rainfall occurring two weeks prior to the collections. Its density showed distinct seasonality, remaining high in monsoon month, attaining the peak in July and crashing down to low to zero level in cool dry months. *An. dirus* recorded overall sporozoite rate of 1.6%, parous rate of 64.7% and was highly anthropophilic (AI 92.3%) and fully susceptible to DDT, dieldrin, malathion and L-cyhalothrin.

O - 5.14

DIAGNOSIS AND SPECIES IDENTIFICATION OF SARCOCYSTIS OF PIGS USING IMMUNOLOGICAL TESTS.

Hemaprasanth
IVRI Campus, Mukteswar-Kumaon, Nainital, U.P. India.

In the present study five serological tests, viz. Single raidal immunodiffusing (SRID), Double immunodiffusion (DID), Immunoelectrophoresis (IEP), Counter immunoelectro phoresis (CIEP) and Rocket immunoelectrophoresis (RIEP) were conducted using *Sarcocystis suihominis* and *S. miescheriana* bradyzoite antigens and their corresponding antibodies (hyperimmune sera) raised in rabbits to evaluate their sensitivity and specificity in diagnosing *Sarcocystis* infection and to study the similarities and differences between these two species. Amongst these five immunodiagnostic tests, CIEP

and SRID gave the most sensitive and specific results as evidenced by the extremely weak reactions in tests using heterologous immune sera. All other tests gave positive reactions with both homologous and heterologous combinations of antigens and antisera. The cross reaction amongst different *Sarcocystis* species in serodiagnostic tests confirm that these species share certain antigenic determinants. However, the weaker reactions observed in tests employing heterologous hyperimmune sera and extra precipitin lines observed in tests employing homologous sera again prove that some of the antigenic determinants of *Sarcocystis* of porcine origin are different and species specific.

O - 5.15

MOLECULAR DISSECTION OF NATURAL IMMUNITY TO MALARIA : IMPLICATION OF RIBOSOMAL PHOSPHOPROTEIN AS A TARGET

Shobona Sharma, S Singh, S Chatterjee, R Sohoni and A Goswami
Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India

It is well documented that gammaglobulins from naturally acquired malaria immune adults can passively confer protection to malaria patients. In an attempt to identify the specific set of antibodies present in such malaria immune persons, we have earlier performed a differential immunoscreen using immune and patient sera samples, and identified several cDNA expression clones of *Plasmodium falciparum,* which react exclusively to the set of immune sera. One such expression clone recognized by a large number of immune sera, has been cloned characterized. It is *P. falciparum* gene homologue of the Po ribosomal phosphoprotein. Several different domains of the Po protein have been expressed as a GST-fusion protein in *E. coli* and antibodies raised against these domains have been used to characterize this protein. Antibodies against different domains of this protein inhibit the growth of *P. falciparum in vitro.* These antibodies do not affect the intra-erythrocytic stages of the parasite, but block the step of erythrocyte invasion by the parasite. IFA results show that in addition to cytoplasmic localization, this protein is also present on the surface of the parasite. Thus, in addition to its role in the ribosomal assembly, this protein appears to play a second role in the host cell invasion. Current results investigating the surface localization and immunogenicity of the different domains of Po protein, as also data pertaining to its relevance in autoimmunity will be presented.

O - 5.16

CLINICAL FEATURES OF FALCIAPRUM MALARIA IN ADULT AND CHILDREN FROM SUNDERGARH, ORISSA

Sanjib Mohanty, Saroj K Mishra, Anita Mohanty, S K Satpathy, J K Patnaik, BS Das
Ispat General Hospital, Rourkela 769 005, Orissa.

The clinical features, complications and mortality due to falciparum malaria is variable in different parts of the globe. Not much is known about the Indian sub-continent. We wanted to study the clinical presentation, incidence complications and mortality in both adults and children amongst patients admitted to the wards of Ispat General Hospital, Rourkela.

Clinical features, complications, morbidity and mortality of 457 patients of falciparum malaria (adult 362 and 95 children) admitted to Ispat General Hospital, Rourkela was studied. There was no significant difference in the symptomatology as regards to duration of fever, headache, vomiting, loose motions, hospital stay, parasite clearance time, fever clearance time or coma recovery time. The main variation in the children is the higher incidence of seizures (7/18 vs 5/57 adults). All other complications are significantly higher in the adults ; viz. cerebral malaria (57 adults, 18 children), renal failure (21 adults, non in children, $p < 0.01$), haemolysis (53 adults and only 2 children $p < 0.001$), Anaemia (i.e. Hb less than 5g / dl in 22 adults, 5 children). The blood transfusion requirement was higher in the children ($p < 0.005$). Multiple complications were frequently encountered in the adults. The common combinations are cerebral malaria with acute renal failure, cerebral malaria with haemolysis or anaemia with haemolysis.

Death occurred in 17 adults and 1 child, ($p < 0.01$). All the patients had cerebral malaria. Out of the 17 adults 16 had multiple complications and the child had cerebral malaria with severe anaemia. The recovery is thus good in the paediatric age group, and carries a high mortality in adults (29.82%).

O - 5.17

NEUROLOGICAL COMPLICATIONS IN PLANSODIUM VIVAX MALARIA

H L Gupta
Department of Medicine, Lady Hardinge Medical College & Assocoated Hospitals.
New Delhi.

It is uncommon to have neurological menifestations in vivax Malaria. Involvement of Nervous System in Malaria occurs in less than 2 percent of cases mainly with Falciparum Infection but rarely it may be due to Plasmodium Vivax.
The persent study comprises of 20 cases of Malaria in which patient developed one or other neurological complications during the course of the disease. In this study only those cases were taken where the smear was positive for P.Vivax. The various Neuro-

logical manifestations observed were mental confusion, coma, convulsions, meningieal irritation, canial nerve palsy Guillan Barre Syndrome,hemiplegia, paraplegia, etc.

O - 5.18

STUDIES ON CERTAIN ASPECTS OF *TRYPANOSOMA THEILERT*, LAVERAN, 1902 - CLINICAL DISEASE SYNDROME IN EXPERIMENTAL CALVES

G Narendra Babu, N Satyanarayanacharyulu, and Md Hafeez
Department of Parasitology, College of Veterinary Science, Acharya N.G. Ranga Agricultural University, Tirupati-517 502 INDIA

Bull calves were used to study the clinical manifestations in *Trypanosoma theileri* infection and these were induced infection with 4 x 10^5 parasites, given subcutaneously. The prepatent period observed was 6 to 7 days and incubation period 5 to 12 days. The clinical manifestations were exhibited from 5th day of post infection and parasitaemia from 6th and 7th days onwards. The symptoms included elevation of body temperature up to 105°F, , dullness, lacrimation with congested mucus membrane and passing loose dung having stray mucous strings. Further, intermedibular oedema, salivation, rough coat, muscular twitching and enlargement of prescapular lymphnodes. During 21st to 30th days of post infection, general condition was much deteriorated with progressive weakness, emaciation, incoordination of movement followed by semicomatose condition and later death supervened. The disappearance of parasites in the blood of experimentally infected calves from 13th to 20 and 26th to 30th days of post infection was noticed. The period of first intermission was associated with swelling of prescapular lymph nodes and second intermission corresponds to the symptoms of incoordination of movements and comatose condition exhibited by the infected calves.

O - 5.19

COMPARISON OF FIELD'S STAIN AND FLUORESCENT DYE (ACRIDINE ORANGE) FOR THE EFFICIENT DETECTION OF PLASMODIUM INFECTION

Parijath N Goswami, Jayshree Patel and Parul Pandit
Division of Microbiology, The Gujarat Cancer & Research Institute, Ahmedabad

Field's stain-a quick staining method for Malarial parasites is the method adapted as a routine practice in the haematology laboratory of the Gujarat Cancer & Research Institute.

In case of scanty parasitaemia, it becomes a tiring job and a tendency to miss the malarial parasites for a microscopist during the procedure of screening. To overcome this, we planned to stain the malarial parasites with a fluorescent dye Acridine orange. Acridine orange has marked affinity for nucleic acids. The RNA components flouresce or-

ange red and the DNA components yellow green. Even though, this method is used to study the virus growth in the host cells and for revealing scanty bacteria in wet films in blood culture we adapted this to detect malarial parasites in the whole blood.

Fifty cases of whole blood from Blood Bank and Haematology Department were screened by both the staining methods i.e. field's stain and fluorescent dye, over a period of 3 months from May '97 to July '97. We also used a new, rapid and easy to perform test called Parasight F, for the evaluation of the samples collected. The results will be analysed statistically and presented during the conference.

O - 6.1

PREDATION ON SNAILS BY AN INDIGENOUS FISH, *SARGOCHROMIS CODRINGTONI*, IN A POND ENVIRONMENT : IMPLICATIONS FOR THE BIOLOGICAL CONTROL OF SCHISTOSOMIASIS

M J Chimbari and H. Madsen
Blair Research Laboratory and Danish Bilharziasis Laboratory, C. Y. 573 Kingsway Road, Harare.

The extent to which *Sargochromis codringtoni* (a known snail eater) can control snail numbers was investigated in cement lined ponds in order to evaluate the fish's potential as an agent for biological control of schistosome intermediate host snails. Snail numbers were significantly reduced over time in ponds where fish were present but remained high in control ponds (without fish). *Bulinus tropicus* of all sizes were vulnerable to predation by fish while both *Melanoides tuberculata* and *Bulinus globosus* of shell heights greater than 10 mm seemed to be less vulnerable to predation. *Bulinus tropicus* was affected more than *Bulinus globosus* by predation probably as a result of its smaller size. In ponds without fish, the density of *B. globosus* was lower than in ponds with fish, while the density of *B. tropicus* was very high in ponds without fish. It is suggested that *B. tropicus* exerted competitive effects on *B. globosus*. Consequently, *S. codringtoni* should probably not be used for control of *B. globosus* in habitats where *B. tropicus* naturally thrives as the latter is more vulnerable to predation by the fish. It was concluded that snails may be significantly controlled by *S. codringtoni* and in order to further investigate this aspect a field trial was recommended.

O - 6.2

HOST-ODOUR SENSITIVITY IN MALARIA MOSQUITOES

Ingrid V F van den Broek
Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

The vectorial capacity of a mosquito population highly depends on its level of anthropophily-the preference to bite human hosts-which is genetically determined. Since host-seeking is mainly activated by olfactory stimuli, mosquitoes are thought to respond to host-specific odours.

We made electrophysiological recordings from antennal olfactory cells during stimulation with host-specific odours in three Anopheline species: *Anopheles gambiae s.s., An. arabiensis* and *An. atroparvus (Diptera, Culicidae)*, which differ as to host preference. *An. gambiae s.s.* is strictly anthropophilic, *An. arabiensis* is opportunistic and *An. atroparvus* is zoophilic. The host odours we applied were five short-chain fatty acids that are found in human sweat; acetic, propionic, butyric, iso-butyric and iso-valeric acid, and three cattle-derived odours : 1 - octen-3-ol, and 3- and 4-methylphenol. We

recorded from receptor cells associated with *Sensilla trichodea* types A1 and A2. The majority of the cells of all three species responded to one or more of these odours at the dose applied (2.5 µg/25 µl ether, in a pipette of 2 ml, injected into a continuous airflow over the antenna during 0.2 s). Fatty acids evoked either increase or decrease of spontaneous spike activity, whereas stimulation with 1-octen-3-ol and the phenols resulted in excitation only. The difference in sensitivity between the species was small but significant and in accordance with their host preference. Cells of *An. gambiae s.s.* and *An. arabiensis* reacted more strongly to fatty acids than the cells of *An. atroparvus*, whereas the latter were more sensitive to 1-octen-3-ol. Currently we are measuring the response to 5 different doses of propionic acid, 1-octen-3-ol and 3-methyl-phenol.

O - 6.3

THE DETECTION AND MONITORING OF ORGANOPHOSPHATE RESISTANCE IN *CULEX TRITAENIORHYNCHUS* THE VECTOR OF JE IN MADURAI SOUTH INDIA

S Poopathi[1], K M Rao[2], N Gopalan[2], N Arunachalam[1], G Baskaran[1] and S P Kandasamy[1]
1. Centre for Research in Medical Entomology (ICMR), 4 Sarojini Street, Chinna chokkikulam, Madurai - 625 002
2. Defence Research and Development Establishment, Gwalior - 474 002 India.

Organophosphate (OP) resistance in the field population of *Culex. tritaeniorhynchus* was studied. *Cx. tritaeniorhynchus* collected from JE endemic areas near Madurai city, were tested for OP-resistance by micro-assay test kit developed by DRDE, Gwalior and further confirmed by WHO bioassay tests. *Cx. tritaeniorhynchus* from CRME laboratory colony were also tested for comparison. Resistance was detected in field population of mosquitoes. In micro-assay test colour development indicated the presence of OP resistance. The bioassay results also showed a high level resistance to organophosphate (Malathion in the field population. The study reveals the occurrence of OP-resistance in *Cx. tritaeniorhynchus* in JE endemic areas in Madurai.

O - 6.4

DELTAMETHRIN - A NEW WEAPON FOR EFFECTIVE CONTROL OF MALARIA

C J Babu and K K Unni
Hoechst Schering Agrevo Ltd., Hoechst Centre, 54-A, Sir M V Road, Andheri (East), Mumbai - 400 093

Malaria control is yet to achieve the desired goals in spite of the best efforts by the programme operators. The reason for such failures are many and one among them is the need for cost-effective and sustainable control method. The general guidelines followed for effective implementation of vector control strategies are :

i) Use of insecticides for selective spraying.
ii) Insecticide impregnated mosquito nets.
iii) Larvicides.
iv) Environmental management.
v) Use of repellents /mats/coils - as personal protection.

In this paper, a new synthetic pyrethroid, Deltamethrin tried in the malaria control programme effectively for the past few years in different states of India, is discussed. The strategy for its judicious use and thwarting the development of resistance to this group, are also being highlighted here.

O - 6.5

P Natarajan
(Abstract not received)

O - 6.6

N J Shetty
(Abstract not received)

O - 6.7

SEASONALITY AND VECTORIAL CAPACITY IN MANSONIOIDES

R K Hazra and A P Dash
Regional Medical Research Centre, Bhubaneswar - 751 016 Orissa, India.

Orissa state is an endemic home for brugian filariasis transmitted by *Mansonia annulifera* and *Ma. uniformis*. The per man hour density of both the species varies between 3.85 to 18.6 and 0.61 to 21.5 respectively with a peak in September. All the developmental stages of *Brugia malayi* were detected in wild caught *Ma. annulifera* and *Ma.uniformis*. Filarial infection in *Ma. annulifera* was found only in four months in a year with a peak infection rate (3.86%) and infectivity rate (0.8%) in October. Computation of correlation coefficients indicated positive correlation between density, infection and infectivity rates. However, negative correlation between infectivity rate and average infective larval load was found in *Ma. annulifera.*

O - 6.8

TRANSMISSION DYNAMICS OF FILARIASIS IS KHURDHA DISTRICT OF ORISSA, INDIA.

N Mahapatra, A P Dash, R K Hazra and A S Acharya
Regional Medical Research Centre, Bhubaneswar - 751 016, Orissa, India

A three year longitudinal study was carried out to quantify the different parameters of filarial transmission in an endemic area of Orissa, India. Parasitological survey revealed microfilaria rate, microfilaria density and median microfilaria density (MFD-50) to be 9.41, 1.23 and 7.33 respectively. The per man hour density of *Cx. quinquefasciatus* varied from 24.2 to 66.0 with a peak in January. Infection rate varied from 0.9 to 27.5%, while infectivity rate ranged between 0.0 and 15.2%. Infectivity rate showed high correlation with microfilaria rate and per man hour density of adult mosquito. Highest number of first stage larvae (L1), second stage larvae (L2) and third stage larvae (L3) per mosquito were found to be 25, 22 and 11 respectively. Average L3 load per mosquito ranged from 1.0 to 7.2. L3 load showed high correlation with microfilaria rate (r=0.845).

O - 6.9

ANOPHELING BREEDING IN INTRADOMESTIC SOURCES AND THEIR MANAGEMENT IN CHENNAI

Indranil Kar[1], Alex Eapen[1], K.John Ravindran[2], R.K. Chandrahas[1] and V.P. Sharma[3]
1. Malaria Research Centre (field station), 1304, Annanagar Western Extension, Mogappair, Chennai - 600050
2. Malaria Research Centre (field station), Epidemic Disease Hospital Campus, Bangalore - 560038.
3. Malaria Research Centre, 22-Shamnath Marg, Delhi-110054

Total of seven mosquito species were found to breed in the domestic breeding sources. *Anopheles stephensi* and *An. subpictus* were found to breed in almost all types of intradomestic breeding sources. Among the culicines *Culex quinquefasciatus* and *Aedes aegypti* were predominant. Larval breeding were controlled through various methods, viz., introduction of larvivorous fishes (*Gambusia afffinis),* source reduction, use of expanded polystyrene beads (EPS) in unused or community sources, mosquito profing of breeding sources etc. Health education helped in generating better cooperation from the community. Intradomestic anopheline positivity ranged between 0.03 to 0.38% and 15.2 - 35.9% in experimental and control areas respectively.

O - 6.10

THE GENETIC STATUS OF THE *ANOPHELES PUNCTULATUS* MOSQUITO COMPLEX : A LOOK AT RIBOSOMAL DNA.

Nigel W Beebe, Allan Saul*, and John T Ellis.
University of Technology, Sydney, Australia.
*Queensland Institute of Medical Research, Brisbane, Australia.

The mosquitoes known collectively as the *Anopheles punctulatus* complex are the most important vectors of human malaria in Papua New Guinea, Irian Jaya (Indonesia), the Solomon Islands and Vanuatu. This complex consists of 10 cryptic species, *An. farauti*

1-7, *An. punctulatus, An. sp. near punctulatus,* and *An. koliensis.* PCR amplification of the ribosomal DNA second internal traanscribed spacer (ITS2) and restriction analysis using a single restriction enzyme resulted in a simple species identification tool. These patterns have been consistent with genomic DNA probes constructed by us for this complex. Upon sequence analysis, the ITS2 region exhibited a G/C content ranging from 61% to 70% and displayed sequence variation between species ranging from 3.2% to 24.3%, suggesting substantial evolutionary distance compared to other cryptic mosquito species. The region adjacent to the 5.8S gene appeared more highly conserved between these species compared to the 3' region adjacent to the 18S, suggesting the 5' region may play a more important role in the RNA processing. Nuclear 18S (2000bp) and mitochondrial 12S (700 bp) genes also under investigation complement this genetic distance and prove to be more useful for evolutionary analysis.

O - 6.11

THE EFFECT OF THE AQUEOUS EXTRACT OF THE CLOVE BUDS ON THE LIFE CYCLE OF MALARIAL VECTOR *ANOPHELES STEPHENSI.*

Ragini Tiwari, A K Sagar and A D Srivastava
Post Graduate Department of Zoology, Bareilly College, Bareilly (U.P.).

The effect of an aqueous extract of clove buds was examined on the different stage of the life cycle of malaria vector, *Anopheles stephensi.* The bioassay was performed according to WHO standards and the corrections where necessary were made by Abbot's formula. A concentration of 3% of the extract caused incomplete hatching of the eggs, while 5% and 10% concentration showed ovicidal and larvicidal activity under laboratory conditions. (Temperature 27 + 2°C and Relative humidity 70 + 5%). These concentrations caused complete mortality of I, II, III and IV instar larvae of the mosquitoes. The changes caused in behaviour, morphology and motility of the adults and larvae were also recorded.

O - 6.12

ANTIBODY BOOST AS A MARKER FOR PROTECTION IN *P. BERGHEI* IRRADIATED SPOROZOITE IMMUNIZATION

Shayama Chatterjee, Pierre Druilhe and Marc Wery.
Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium & Institute Pastcur, Paris, France.

Immunization with irradiated sporozoites of *P. berghei* protects rodents to a live sporozoite challenge. This protection is multifactorial in nature, dependent on the schedule, dose, route of immunization of irradiated sporozoites as well as the host species used for immunization. Protection is obtained only when the irradiated sporozoites are injected intravenously, indicating that the transformation of irradiated sporozoites into liver stages is essential, and therefore to the liver stage parasites as being crucial for

inducing host protection. Liver stage parasites developing from irradiated sporozoites do not attain maturity but are blocked in the host liver as immature forms. Sera antibodies generated in immunized C57BL6 mice show reactivity to young liver stage parasite *in vitro* at titers of 100. Upon live sporozoite challenge of these immunized mice a boost in liver stage specific-antibody responses specifically of the IgG_{2a} & IgG_{2b} isotypes is observed, with titers reaching 4000. Associated with high IgG titers, a significant inhibition of liver stage development is also mediated by these sera antibodies *un vitro.*

Two questions arise from these observations : (1) **What is the fate of live sporozoites developing in irradiated sporozoite immunized hosts ?** Repeated immunizations with a large number of irradiated sporozoites generates anti-liver stage responses which are low, however indicate T-B cell interactions and generation of antibodies and memory T cells to antigens expressed by blocked liver forms. Upon live sprozoite challenge with a few sporozoites only, a high antibody boost is observed. It is possible that novel, potent B cell epitopes are expressed by liver forms developing from live sprozoites, which in cooperation with memory T cells generated during immunizations are responsible for this antibody boost.

(2) **What is the function of the IgG_{2a} & IgG_{2b} antibody isotypes generated during challenge infections ?** A role of the T_{h1} immune effector arm in the induction of protection in this mice strains is indicated. We
aim to study the proliferative responses of immune splenic lymphocytes to liver stage antigens and the corresponding cytokines (specially IL-4, IL-12, IFN-g, IL-10) secreted into stimulated culture supernatants. A comparison of the above observations in immune versus non immune hosts can shed light on our hypothesis that the antibody boost observed upon sporozoite challenge of immunized mice is a marker for protection.

O - 6.13

ROLE OF VECTOR *CX. QUINQUEFASCIATUS* IN THE TRANSMISSION OF FILARIASIS IN TRIBAL AND NONTRIBAL AREAS OF BANKURA DISTRICT, WEST BENGAL, INDIA

S K Rudra and G Chandra
Department of Zoology, University of Burdwan, 713 104, West Bengal, India

Bankura district of West Bengal is endemic for bancroftian filariasis. Most of the filarial works on epidemiology and vector biology in West Bengal were confined to Calcutta and its suburbs. Practically no work was made in tribal field regarding the vector status of bancroftian filariasis. The present study has been designed to know the vector bio-

logical aspects of filariasis in both tribal and nontribal fields of Bankura district of West Bengal on a comparative basis. Indoor-resting mosquitoes were captured from fixed human habitations of tribal and nontribal areas in alternate week from August, 1994 to July, 1995 employing a total of 96 man-hours. Different parameters like per man hour density, infection rate and infectivity rate of incriminated vector, *Cx. quinquefasciatus* were 9.91%, 2.05% and 0% respectively in tribal area and 45.6%, 4.32% and 0.50% respectively in nontribal area. All the above parameters of vector mosquito were found very low in tribal area than those of nontribal area. Potential Transmission Index and Annual Transmission Potential were also calculated in both the fields and found higher in nontribal area.

Epidemiological study in the same area revealed that the tribes suffer less from filariasis than the nontribes. It may be pointed out from the present study that different aspects associated with the vector play vital role in reduced filarial prevalence among tribal population.

O - 6.14

A K Kumar
(Abstract not received)

O - 6.15

ANOPHELES MINIMUS TRANSMITTED MALARIA IN ASSAM, INDIA

P Dutta and J Mahanta
Regional Medical Research Centre (ICMR) Post Box 105, Dibrugarh-786 001, Assam, India

The studies conducted in some parts of upper, middle and lower Assam covering different biotopes reveal that the areas having foothill forest ecosystems are highly malarious showing slide positivity rate ranged from 22.2-50.8 and *Plasmodium falciparum* is the predominant species forming 70-97 percent of the total positives along with a considerable degree of chloroquine resistane. *Anopheles minimus* has been incriminated with high sporozoite rate from majority of study areas endowed with foothills and forests where persistent transmission of malaria is observed. In contrast, the plain areas or valleys where no forests or foothills are located, are almost free from malaria due to the absence of the major malaria vectors.

An. minimus is found to be exclusively endophilic pertaining to rest indoors of dark mudplastered houses and being highly susceptible to conventional insecticides, the spray of DDT or other insecticides is still effective in controlling malaria transmitted by *An. minimus* in Assam. Therefore, its distribution seems to be patchy, only in some pockets of low or no DDT coverage and also it is observed to have either disappeared or has become scarce in many of its old areas of prevalence. Further, the proper use of bednets was found to be significantly effective in reducing transmission in highly endemic ar-

eas.

O - 6.16

THE FXA-DIRECTED ANTICOAGULANT FROM THE MOSQUITO, *AEDES AEGYPTI*, IS A MEMBER OF THE SERPIN SUPERFAMILY

Kenneth R Stark and Anthony A James
University of California, Irvine USA.

Mosquito salivary glands produce a number of proteins that are secreted during feeding to inhibit mammalian hemostasis and facilitate bloodfeeding. Until recently, little was known about the biochemical and molecular properties of mosquito anticoagulants. We recently demonstrated that culicine mosquitoes have FXa-directed anticoagulants and anopheline mosquitoes have thrombin-directed anticoagulants. The FXa-directed anticoagulant from the yellow fever mosquito, *Aedes aegypti,* was shown to be a revesible, non-competitive inhibitor of FXa that was found only in female salivary glands. This activity was partially purified by cation exchange chromatography and was shown to correspond to a 54kDa protein by SDS-PAGE analysis and peak activity profiles. This protein was further purified by preparative SDS-PAGE and subjected to internal protein sequencing. From the obtained pcptide sequences, degenerate oligonucleotides were designed to screen a female, salivary gland cDNA library. The resulting cDNA encodes a 415 amino acid protein with a predicted molecular weight of 47.8 kDa and contains the five sequenced peptide including two that were not utilized for the cDNA screen. Although the N-terminus was blocked, hydrophobicity analysis predicts a 19 amino acid leader peptide typical for secreted proteins. Northern analysis illustrates that the mRNA is expressed only in female salivary glands. Bacculovirus-expressed cDNA has the expected FXa-directed anticoagulant activity. Sequence comparisons illustrates similarities to the serpin superfamily of serine protease inhibitors. Although the anticoagulant has structural similarities to serpins, it does not form an SDS-stable complex with its cognate serine protease (FXa) and thereby is a novel member of this family. This is the first anticoagulant isolated from a hematophagous arthropod shown to be a serpin-like molecule and represents a novel adaptation of bloodfeeding.

O - 6.17

RESISTANCE MANAGEMENT IN MALARIA VECTOR CONTROL - THE ROLE OF BENDIOCARB

J Goose
AgrEvo Environmental Health, Cambridge, England.

Insecticide resistance is the most serious threat to malaria vector control and most countries have experienced the problem to some degree since large scale programmes were introduced almost 50 years ago. Resistance to BDI developed soon after its introduction and fortunately replacement insecticides were found progressively from the

organophorus carbamate and later the synthetic pyrethroid groups unit at the present time synthetic pyrethroids are used in most control programmes.

However, there is no reason to doubt that the pyrethroids will not also suffer the development of resistance and resistance management strategies to preserve available insecticides are vital. Bendiocarb is a carbamate with a different mode of action from the pyrethroids, which gives up to 6 months residual control at dose rates up to 400 mg a i/ m^2. Like the pyrethroids it does not stain sprayed surfaces, it has no smell and it controls cockroaches and bed bugs. The product is thus very acceptable, and it has an excellent reputation for effective and safe vector control.

O - 6.18

DEVELOPMENT OF MICROBIAL LARVICIDES FOR EFFECTIVE CONTROL OF MALARIA VECTORS

Brian E Melin
Abbott Laboratories, N. Chicago, IL 60064, USA

With the increasing development of insect resistance of chemical larvicides, microbial larvicides based upon *Bacillus thuringliensis israelensis* and *Bacillus* sphaericus are being used effectively with greater frequency in many parts of the world. Research to develop more effective formulations specifically designed for *Anopheles* spp. has been the key to the recent successes.

Three promising formulations which have been evaluated in the USA have shown improvement over existing formulations and are now ready for expanded field trials in other countries.

O - 6.19

S K Mohanty
(Abstract not received)

O - 6.20

S Singh
(Abstract not received)

O - 6.21

SUITABILITY OF *DUGESIS* SP. (PLATYHELMINTHES : TURBELLARIA) FROM DELHI, AS A BIOLOGICAL CONTROL AGENT OF MOSQUITOES

Raminder Kaur
National Institute of Communicable Diseases, 22, Sham Nath Marg, Delhi - 110 054

Dugesia species have shown great potential as biological control agents of mosquitoes in USA. In India their potential had not been studied for exploitation in this regard.

A *Dugesia* sp. (confirmation of the specific identity awaited from the taxonomist of the group), caught locally from Delhi ridge, was successfully cultured and reared in laboratory (the colony is in its third year of succession) and its larvicidal potential studied under laboratory conditions. The species shows great promise as a suitable biological control agent of mosquitoes since it is easy to mass produce (grows exponentially, upon inoculation in well oxygenated water where food is not limiting and stressful factors such as bright light are eliminated) and is an efficient killer i) as a predator (prefers mosquito larvae) and ii) indirectly by trapping a greater number of prey in its sticky mucus.

The presence of other aquatic organisms viz., sponges, ostracods and fish did not show any deleterious effect on their population. However the recommended larvicidal dosages, used under the control programmes, showed deleterious and devastating effect on the species, which disintegrates within an hour of the exposure to the concerned dosage. Even traces of these wiped out the population directly through exposure or indirectly through consumption of treated but live larvae.

O - 7.1

IMMUNOMODULATING ROLE OF GLYCYRRHIZIN IN EXPERIMENTAL MURINE CEREBRAL MALARIA

M L Dubey, B Mahajan, R C Mahajan and N K Ganguly
Departments of Parasitology and Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh - 160 012, India

An experimental murine model for cerebral malaria was developed by infecting BALB/c mice with 10^6 PRBCs of *Plasmodium berghei* ANKA strain. Infected animals were treated with Interferon-g (IFN-g) inducer glycyrrhizin (GL, 50mg/kg body weight) and/or subcurative dose of chloroquine (CQ, 4mg/kg body weight). Histopathology of the brain sections, parasitaemia in peripheral blood and flow cytometric analysis of the CD4+ and CD8+ T cells, alongwith the level of IFN-g were studied on 3, 6 and 8 days post infection (dpi).

P.berghei ANKA infected untreated mice died on 8 dpi with parasitaemia as high as 50%. Brain sections revealed petechial and occasional gross haemorrhages and blood vessels full of parasitized erythrocytes (PRBCs) alongwith infiltrating mononuclear cells. CD4+:CD8+ T cells ratio was increased throughout the course of infection. Whereas in group treated with the combination of GL + CQ, parasitaemia on 8 dpi was significantly lower as compared to the untreated animals or animals treated with only CQ or only GL. In these animals brain sections were almost devoid of PRBCs and CD4 + : CD8 + T cells approached near normal earlier (on 6 dpi) compared to only CQ or only GL treated group of mice. IFN-g levels were also significantly higher on 6 dpi in CQ + GL treated group thus suggesting that GL may be playing a role in controlling the course of disease in *P. berghei* infected mice, probably by modulating the immune response through induction of IFN-g production.

O - 7.2

PLASMODIUM BERGHEI INDUCES APOPTOSIS IN SPLENIC LYMPHOCYTES : POSSIBLE ROLE IN IMMUNOSUPPRESSION

Babita Mahajan, M L Dubey, G Bhatti, R C Mahajan and N K Ganguly
Departments of Parasitology and Experimental Medicine & Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh-160 012, India.

An experimental murine model for cerebral malaria was developed by infecting BALB/c mice with *P. berghei* ANKA strain. Histopathology of the brain sections, flow cytometric analysis for CD4+ and CD8+ T cells *in vitro* proliferative response to plasmodial antigen alongwith the level of spontaneous and antigen induced apoptosis in cultured lymphocytes, were studied on 3, 6 and 8 days post infection.

Histopathology of brain sections revealed petechial haemorrhages and blocking of the

brain capillaries by parasitized RBCs and adhering mononuclear cells. CD4+ : CD8+ ratio was increased throughout the course of infection.

T-lymphocytes showed low or no response to plasmodial antigens and mitogens. Flowcytometric, electron microscopic and DNA analysis revealed high level of apoptosis of splenic lymphocytes throughout the course of infection. These findings suggest probable role of apoptosis in disturbed CD4+ : CD8+ T cells and these lymphocytes instead of proliferation are eliminated by apoptosis which further may be the cause of immune unresponsiveness to plasmodial antigens and general immunosuppression seen in malaria.

O - 7.3

URBAN MALARIA IN GUJARAT STATE, INDIA: EPIDEMIOLOGICAL STUDY AND MALARIA ACTION PLAN FOR AHMEDABAD CITY

R S Yadav,[1] V P Sharma[2] and R M Bhatt[1]
1. Malaria Research Centre, Civil Hospital, Nadiad 387 001, India;
2. Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India

In recent years there has been a major concern for the rising problem of urban malaria in India. As a true concern on urban health problems, one of which is malaria, in 1996 the WHO gave a call for "healthy cities". In 1995 about a fourth of 0.2 million malaria cases in Gujarat were reported from 68 urban areas comprising 25% (10 million) population. This proportion is projected to reach 50% mark during next 2 decades with a major contribution by *Plasmodium falciparum.* In view of this we took up a study on malaria in Ahmedabad city (core population over 3 million) in 1996 to develop a malaria action plan aiming that it would have regional and national relevance. Major components of the study were : geographical reconnaissance for vector breeding habitats; study of vector bionomics; assessment of knowledge, attitude and practices related to mosquito-borne diseases among the people; malaria morbidity estimation and review of the existing surveillance mechanism to develop a Malaria Information System; assessment of current vector control measures, manpower requirement, training needs and role of the non-health sectors in containment of the problem. The study is in an advance stage. From the experiences it is felt that there is a great need for appropriate information, education and communication for participatory management of the problem. Integration of bioenvironmental methods of control and improvement in building byelaws are desired. Environment and health impact assessment should become an integral part of the urban development planning so that necessary preventive health measures are incorporated at the right stage. These issues and progress of the study will be discussed.

O - 7.4

STUDY OF PATHOGENIC MEDIATORS IN MALARIA INFECTION PLASMA

N D Karunaweera[1], K N Mendis[1] and R Carter[2]
1. Department of Parasitology, Faculty of Medicine, Colombo.
2. $ ICAPB, University of Edinburgh, U.K.

Factors produced by the host and the parasite during an infection of malaria are known to mediate the clinical symptoms of acute infections. We have previously demonstrated that plasma taken during the peak of *P. vivax* paroxysm inactivates gametocytes of *Plasmodium* rendering them non-infective to mosquitoes. The factors mediate which this effect have implicated in the mechanisms underlying a paroxysm with which this event coincides.

In the present study, we have tested the effects of malaria infection plasma, on two continuous human cell lines (umbilical vein endothelial cells and melanoma cells) which are closely related to endothelial cells lining the blood vessels. The effects were assayed by culturing the cells at 37°C in medium containing test and non-malaria plasmas and testing the viability of cells following incubation. Compared to controls, all of the plasma samples from 15 patients tested for their ability to metabolize a chemical dye (MTT) showed an inactivation of the mitochondrial dehydrogenase enzyme in these cells indicating impaired viability, when assessed after 3 hours in culture. However, after 24 hours in culture cells grown in malarial plasma had regained their ability to metabolize MTT, as well as those grown in control plasma, indicating that cellular enzyme inactivation was transient. This effect was seen in plasma taken both during, and between paroxysm but not in plasma samples taken during convalescence, hence, the active molecule(s) are present in plasma only during the acute phase of the infection. The cellular enzyme inactivation caused by the malaria infection plasma was not reversed by the addition to the malarial plasma, of antibodies against i) cytokines TNF- α, GM-CSF, IFN - τ, IL-16, IL-2, IL-4 or IL-6 even in combination or ii) a parasite schizont extract. Neither was the enzyme inactivation elicited by the addition of recombinant cytokines TNF-alpha or IL-6 or of a parasite extract to normal plasma, even in combination. Testing of different fractions of malaria infection plasma obtained following ultra centrifugation indicated that the factor(s) mediating this effect are water soluble. Further characterization has indicated that the molecular weight(s) are > 30 KDa and the active molecule(s) are heat labile. We conclude that the mediator(s) of the transient inactivation of cellular enzymes comprise large, heat labile, water soluble molecule(s) which are likely to be protein in nature and produced during acute malaria infection by the host.

O - 7.5

PREVALENCE OF GASTRO-INTESTINAL PARASITIC INFECTIONS IN DAIRY CATTLE AND BUFFALOES IN AND AROUND TIRUPATI ANDHRA PRADESH - INDIA

C Sridevi, Md Hafeez and N S Charyulu
Department of parasitology, College of Veterinary Science, Acharya N.G. Ranga Agricultural University, Tirupati 517 502 (A.P.) INDIA.

In the present study, 780 cattle and 694 buffaloes were screened. The overall per cent of incidence in cattle and buffaloes was 44.23 ± 1.94 and 37.75 ±1.17 respectively. Among the six places under study the incidence was more in Srinivasa Mangapuram (52.3%) and least in Tirupati (40.97%). In cattle, incidence of *Strongyle* sp.(19.4%) was higher and *Fasciola* sp. (0.9%) was lower, whereas in buffaloes the incidence of *Balantidium coli* (21.8%) recorded was more and *Fasciola* sp. (1.14%) was less.

The overall incidence of intestinal parasitic infections was higher during rainy season. Incidence of *Strongyle* sp. (11.6%) in cattle and *B.coli* (14.1%) in buffaloes was more prevalent during rainy season. The incidence of infection with *Trichuris* sp. (3.5%) was maximum during winter and summer seasons in cattle whereas in buffaloes the infection of *Amphistome* sp. (3.4%) was more during winter and *Strongyle* sp. (6.9%) during summer.

Incidence of overall gastrointestinal parasitic infections among infected was highest in above 3 years age group of cattle (71.6%) followed by 1-3 years (19.1%) and 0-1 year old groups (9.3%). In buffaloes the incidence rate of 61.1, 25.2 and 13.7 per cent was observed respectively in similar age groups.

O - 7.6

ANOPHELES STEPHENSI BUILD-UP AND ACCELERATED MALARIA TRANSMISSION IN THE POST BIO-CONTROL INTERVENTION PHASE IN CANDOLIM PHC OF GOA, INDIA.

Ashwani Kumar[1], V P Sharma[2], P K Sumodan[1] and D Thavaselvam[1]
1. Malaria Research Centre (ICMR), Field Station, Directorate of Health Services Building, Campal, Panaji- 403 001, Goa, India.
2. Malaria Research Centre (ICMR), 20 Madhuban, Vikas Marg, Delhi-110 092, India.

Coastal villages of Candolim Primary Health Centre witnessed rampant construction activity and aggregation of migrant labour leading to *Anopheles stephensi* build-up and out break of malaria in 1993 (Cases : 586, SPR : 10.6%, API : 12.4) and 1994 (Cases : 1431, SPR : 17.3% API : 30.4). With the selective introduction of biological vector control measures comprising of larvivorous fish *Aplocheilus blocki* and *Bacillus*

thuringiensis hisraelensis in the breeding habitats of *An. stephensi* coupled with early case detection and prompt treatment, the malaria incidence declined sharply in 1995 (Cases : 263, SPR : 7.4%, API : 5.6). Withdrawal of these measures in 1996 led to abrupt vector build-up and accelerated malaria transmission as 3481 malaria cases including 371 *Plasmodium falciparum* cases were reported with a SPR of 26.4% and API fo 73.9%. Well supervised re-introduction of these biological vector control measures is recommended to check malaria transmission in Candolim PHC and similar situations.

O - 7.7

SEQUESTERED PARASITE POPULATION DYNAMICS IN CEREBRAL MALARIA

Michael B Gravenor, Michael Boele van Hensbroek and Dominic Kwiatkowski.
Department of Paediatrics, Institute of Molecular Medicine, University of Oxford, Oxford and Department of Paediatrics, Academical Medical Centre, Amsterdam, The Netherlands.

Measurements of parasite load in malaria infections rely on sampling parasites from peripheral blood smears. In the case of *Plasmodium falciparum,* this method ignores mature parasites that sequester within the microvasculature and do not circulate in the peripheral blood. Parasite sequestration is thought to be central to the pathogenesis of severe malaria, but investigation of this problem is greatly limited by the lack of a quantitative measure of sequestered parasites. Using a mathematical model, we described a method of estimating this hidden population from sequential observations of peripheral parasitaemia, and analyse sequestered parasite population dynamics under different drug regimes.

Applying the model to clinical data from 217 Gambian children undergoing treatment for cerebral malaria we find that, although artemether clears peripheral parasitaemia more rapidly than quinine, the clearance of sequestered parasites is similar for the two drugs. Comparing observed parasitaemia and estimated sequestered mass by logistic regression, sequestered mass was found to be the more direct predictor of fatal outcome. These results suggest that the behaviour of the sequestered parasite population is of critical importance for clinical management and may explain why artemether provides little advantage over quinine in reducing mortality despite its rapid effect on circulating parasites.

O - 7.8

HUMAN TNF ALLELE POLYMORPHISMS ASSOCIATED WITH RISK OF SEVERE DISEASE IN *PLASMODIUM FALCIPARUM* MALARIA.

S Premawans[1], J Wattawidanage[1], K L R L Perera[1], A Munesinghe[1], S Bandara[1], R Wickremasinghe[1], H Alles[1], KN Mendis[1] and R Carter[2]
1. Malaria Research Unit, University of Colombo, Sri Lanka.
2. Division of Biological Sciences, University of Edinburgh, Scotland, The United Kingdom.

The distribution of human host genetic polymorphisms have been investigated at the TNF-alpha and TNF-beta loci with respect to the degree of severity of *P. falciparum* malaria disease in a Sri Lankan population. Two categories of patients viz. 118 of uncomplicated malaria (UC), 35 severe and complicated malaria (SC) along with 84 healthy controls (HC) were subjected to this study. The severity of disease in the patients was determined by a previously validated numerical scoring system based on criteria described by the World Health Organization for characterizing severe and complicated *P. falciparum* malaria. The patients were typed for their TNF allelic types; for TNF-alpha*1 and *2 and TNF-beta *1 and *2 alleles using PCR and allele-specific oligonucleotide probing. It was found that the frequency of TNF-alpha *2 allele was significantly higher in SC group than that in UC or HC groups. The heterozygous genotype TNF-alpha *1, *2, was associated with a two to three-fold increased risk of severe malarial disease compared to the TNF-alpha *1, *1 homozygote. Thus, an increased risk for severe and complicated malaria in this population occurred in the heterozygous state, TNF-alpha *1, *2 and not just in the TNF-alpha *2, *2 homozygous state as reported by McGuire *et al* in 1994. In the present study the increased risk for severe malaria disease associated with the TNF-alpha *2 allele occurred, however, only in the presence of the TNF-beta *2 allele. Likewise, increased risk of severe disease in the presence of TNF-beta *2 allele occurred only in the presence of the TNF-alpha *2 allele. These observations suggest the possibility of an epistatic relationship between the TNF-alpha *2 and TNF-beta*2 alleles in conferring high risk for severe and complicated *P. falciparum* malarial disease.

O - 7.9

ASSOCIATION BETWEEN ABO BLOOD GROUPS AND PROTECTION AGAINST SEVERE AND COMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

P P S L Pathirana, H K Alles, M K Perea, KN Mendis, and SM Handunnetti
Malaria Research Unit, Faculty of Medicine, University of Colombo, Sri Lanka.

The ABO blood group composition in two clinical groups of *Plasmodium falciparum*-infected patients having severe & complicated (SC) and uncomplicated (UC) malaria infections were analyzed in this study. The ABO blood group composition of the SC

group of patients (n=61) was significantly different from that of the UC group (n=160) ($p < 0.001$; $\gamma^2=16.46$) as well as from a population sample (n=50,320) taken from a community survey ($p<0.0001$; $\gamma^2=28.15$). The distribution of ABO blood groups in the uncomplicated malaria patients and population control was comparable; the relative representations of blood groups AB and O were almost identical and of the blood groups And B were similar in these two groups.

The occurrence of O blood group in the SC group (23%) was significantly lower compared to that in the group of uncomplicated malaria patients (47%; $p=0.001$; $\gamma^2=10.51$) and in the general population sample (45.6%; $p<0.001$; $\gamma^2=11.66$). There was also a significantly higher incidence of the AB blood group in the SC group (18%) compared to that of either the UC group (5%; $p<0.01$; $\gamma^2=9.55$) or the population control group (5.6%; $p<0.0001$; $\gamma^2=18.05$). These finding suggest that i) the O blood group may be protective against the development of severe and complicated diseases and/or ii) that the AB blood group confers susceptibility to the development of severe and complicated disease in *P. falciparum* malaria. The incidence of blood groups A was higher in the SC group compared to the UC group although this difference was not statistically significant. There is no evidence from this study to suggest that the ABO blood group system affects susceptibility to infection with *P. falciparum* malaria, however, the data suggest that it does influence significantly, the susceptibility of persons with *P. falciparum* infection to progress to a severe and complicated state.

O - 7.10

FACTORS AFFECTING CASE FATALITY RATES IN PATIENTS FROM ENDEMIC AND NON-ENDEMIC LOCATIONS OF 2 SOUTHERN ASIAN COUNTRIES (SRI LANKA AND MYANMAR)

H K Alles+, K N Mendis+ and R Carter+#

+ University of Colombo, Faculty of Medicine, Department of Parasitology, Malaria Research Unit, Kynsey Road, Colombo 8, Sri Lanka, # University of Edinburgh, Division of Biological1 Sciences, Institute of Cell, Animla and population Biology, West Mains Road, Edinburgh EH9 3JT U.K.

Malaria case incidence records of 4 endemic regions in Sri Lanka viz.

Anuradhapura, Polonnaruwa, Kurunegala and Uva for 4 years (1991 to 1994) and 5 States/ Divisions in Myanmar viz. Shan, Rakhine, Kachin, Kayah and Kayin for 3 years (1991 to 1993) were analyzed for this purpose. The mean case fatality rats of P. falciparum malaria (death per 10,000 cases) in the endemic zones metioned above in Sri Lanka and Myanmar were found to be 1.3 and 1.8 respectively, the Contrast, in Traveller patients in Colombo, the capital of Sri Lanka, where malaria is not endemic, the cas fatality rate was about 100 fold higher. This difference is likely to be due to some extent to a degree of protective immunity in the endemic population. It is unlikely, however, that immunity is the sole, or even main reason as the following analysis suggest. (i) Mortality rates in the population increase in proportion to the estimated Pfalciparum inoculation

rates in Sri Lanka (Range 0.04 to 0.27) and Myanmar (Range 0.53 to 4.1) which would ne the case if there was significantly greater acquired immunity at higher inoculation rates as occurs in much of tropical Africa. (ii) The data on actual treatment practices in Sri Lanka show that on an average, most malaria patients in endemic area sthe diagnosed the treated earlier (by the third day os symptoms) than the traveller patients in Colombo who receive treatment much later (by the seventh day of symptoms). The tratment on third day of symptoms was beneficial to endemic patients because the mean time taken for an untreated infection to lead to the 2 common severe and complicated states in these countries was 4.9 days for cerebral malaria and 7.8 days for multiple organs/system dysfunction syndrome.

Thus the rapid diagnosis and treatment received by the endemic patients correlate with the very low case fatality rates while the delayed treatmetn in the traveller patients correlate with the much higher case fatality rates.

O -7.11

RISK FACTORS OF *TRICHURIS TRICHURA* INFECTION : EXPERIENCE FROM A RURAL COMMUNITY IN ASSAM

Kanwar Narain, S K Rajguru and J Mahanta
Regional Medical Research Centre, N.E. Region ICMR, Post Box # 105, Dibrugarh 786001, Assam, INDIA.

Stool samples were collected from 222 residents in a rural area in Assam, India and were examined for intestinal parasites. Data on many potential risk factors of infection was obtained by administering a questionnaire to the head of the family and also by observational methods. The results show that 15.3% of individuals were found infected with *Trichuris trichura* and did not show household aggregation. Age, sex, income, number of persons living in the households, type of flooring and pattern of latrine use did not seem to be associated with the prevalence of *Trichuris* infection in the study village. However, the number of cattle owned by households seemed to be a major risk factor for *Trichuris* infection (odds ratio = 3.17; 95% confidence interval = 1.1 to 8.9). Regression analysis between the number of cattle owned by the households and *Trichuris* prevalence also confirmed this view. About 47% of *Trichuris* infected persons showed abdominal distress and the number of bowel movements per week were significantly more as compared to uninfected persons. Infection due to *Ascaris* and *Trichuris* was significantly associated (chisquare = 4.8; $p < .05$).

O - 7.12

EXPERIMENTAL MODEL OF INTRAVAGINAL TRICHMONIASIS INDUCED WITH *TRICHOMONAS VAGINALIS* STRAINS ISOLATED FROM SYMPTOMATIC AND ASYMPTOMATIC WOMEN

I Gupta, M Kaur, N K Ganguly, R C Mahajan and N Malla
Postgraduate Institute of Medical Education & Research, Chandigarh-160 012, India.

An experimental model for the development of intravaginal trichomoniasis was attempted by the intravaginal inoculation of two axenised *Trichomonas vaginalis*(TV) strains isolated one each from symptomatic and asymptomatic women. The infection could be successfully induced in 4-6 wks old female BALB/c mice weighing 22-25g by giving 50µl of Estradiol velerate (10 mg/ml) subcutaneously followed by intravaginal inoculation of *Lactobacillus acidophilus* for two consecutive days ($2x10^8$ organisms/mouse). After one week second dose of Estradiol valerate was injected followed by intravaginal inoculation of 50µl of *T. vaginalis* suspension (10^6 organism/ml) daily for two consecutive days. Two groups of five mice each were injected with *T. vaginalis* strains isolated from asymptomatic and symptomatic women. After 3rd day post inoculation till 7th day, vaginal washes were checked daily from each mouse for *T. vaginalis* and inoculated in triplicate into TPS-1 medium. The inoculated culture was examined daily till 7th day post inoculation for presence of *T. vaginalis* by direct microscopic examination. *T. vaginalis* was observed on 3rd-4th post inoculation day in vaginal wash by direct microscopic examination and in culture media two to three days after inoculation. The mice fell sick on 6th-7th post inoculation day and were sacrificed. The vagina, cervix and uterus were dissected out and were submitted for histopathological examination. This model for intravaginal trichomoniasis may serve useful purpose for the study of pathogenesis, drug interaction and local mucosal immune response.

O - 7.13

DECENTRALISED APPROACH TO MALARIA CONTROL IN INDIA

Neeraj Dhingra, G P Dhillon and Shiv Lal
Directorate of National Malaria Eradication Programme, 22, Shamnath Marg, Delhi - 110 054.

Malaria Control in India has been carried out by Directorate of National Malaria Eradication Programme since its inception in 1958 on a vertical approach. The technical strategies were formulated at the centre and implemented by the states and districts. Participation from the states/districts and especially community was minimal, thereby leading non-acceptance of many of the interventional strategies for malaria control. Experimental approach of involving the districts and also the representatives of the community and other sectors was initiated in four selected districts of Andhra Pradesh, Maharashtra, Orissa and Rajasthan in the months of May-June 1996. Social Participation Workshops involving the district planners (administrators, health authorities), com-

munity groups - members of prominent non-governmental organisations, panchayats, women representatives and allied sectors like the social welfare and tribal development were carried out in each district. These Workshops were followed by planning exercises for making a district action plan for malarial control.

It has been observed that the workshops undertaken just before the transmission season generate high level of awareness and enhance the acceptance of the various malaria control interventions. The positive response of the community to take on some of the responsibilities of malaria control is an encouraging step towards the "bottoms up" approach for planning malaria control at district level.

O - 7.14

VISCERAL LEISHMANIASIS CONTROL IN INDIA - AN APPROACH THROUGH PRIMARY HEALTH CARE SYSTEM.

N B L Saxena
Directorate of National Malarial Eradication Programme, 22-Sham Nath Marg, Delhi - 110 054, India.

India is one of the oldest known endemic area of Visceral Leishmaniasis and the available records and the occurrence can be tracked back to 1824-25 when a disease like kala-azar was described in Jessore as "*Jwar Bukhar*". The disease is highly endemic in several parts of the country, all along the eastern coast and the northern India. States like Assam, Bihar, Uttar Pradesh, West Bengal, coastal Orissa, Andhra Pradesh and TamilNadu were among the highly endemic areas. Malaria control coupled with treatment through kala-azar clinics brought down the incidence to a negligible level by sixties. However, disease resurgence was reported in seventies. In seventies initially in four districts of Bihar and gradually the disease became highly endemic in entire northern Bihar and several districts of Wast Bengal besides a few pockets in southern Bihar. The organised control programme is initiated in 1991 and intensified in 1991-92 through existing Primary Health Care System. The decentralized planning approach was adopted for block level and district level planning which mainly included the national level planning and implementation under the supervision and guidance at the Central and State level agencies. The implementation of strategy resulted in effective control of disease within the first two years of its implementation registering a decline of 67 per cent in morbidity and 73 per cent in mortality in 1994 as compared to 1992. The disease trends, the strategy of planning and implementation of Visceral Leishmaniasis control in India are discussed in this paper.

O - 7.15

LYMPHATIC FILARIASIS CONTROL THROUGH MEDICATED SALT : A SUCCESSFUL EXPERIENCE OF COMMUNITY INVOLVEMENT

*Appavoo, N C, **Srivastava, P K, ***Saxena, N B L, Shiv Lal
* Directorate of Public Health and Preventive Medicine, Chennai-6
** Regional Office for Health & Family Welfare, Chennai-90
*** Directorate of National Malaria Eradication Programme, Delhi-54

Success of DEC medicated salt is known through various limited scale trials in different parts of the World. In Kiliyur village of Villupuram district in Tamil Nadu (India), a six year study from 1989 to 1995 was taken up for lymphatic filariasis control through DEC medicated slat with an objective of studying the community response and feasibility of voluntary community involvement in filariasis control. The six years data reveal a very hgih level of community acceptance with highly significnat decline in filariametric indices. Microfilaria rate declined to zero level from pre trial level of 16.76 per cent. Infection rate also declined from 0.76 per cent to Zero besides compatible decline in other indices. The status is being maintained upto two years after withdrawl of medicated salt from the area and further monitoring is continued. The success of the trial lead to a resistance from the area and further monitoring is continued. The success of the trial lead to a resistance fromt he community for withdrawl and reintroduction is being demanded.

O - 7.16

SELECTIVE SPRAY TREATMENT FOR MOSQUITO CONTROL - A DIFFUSION MODEL

P Basker,[1] M Ganesan[2] S Ganesan[3] and N C Appavoo[4]
1. Institute of Vector Control & Zoonoses, Hosur-635 126, Tamil Nadu.
2. Senior Entomologist, Zonal Entomological Team, Trichirappalli, T.N.
3. Research & Development, Elnet, Hosur-635 126 T.N.
4. Director of Public Health & Preventive Medicine (Malaria & Filaria), Chennai-600 006, Tamil Nadu.

Selective spray treatment for mosquito control of a semi infinite pool extending from the plane boundary x=0 to x= α is considered. At the time t=0 a spray chemical S is brought into contact at the plane x=0 of the pool, the surface concentration of the chemical being C_{s0}. Spray chemical is conceived to spread on the surface with time and in space. C_{sl}, a critical limit of spray concentration is envisaged such that mortality of mosquito larvae is 100% if the spray concentration C_s exceeds C_{sl} for C_s less than C_{sl} death of larvae is not guaranteed. Thus the spray would be effective for a period t_1 in the space 0 to say x^1 if the concentration obeys the constraint $C_s(x,t) \geq C_{s1}$ for $x \leq x_1$ and $t \leq t_1$. If x_1 and t_1 can be established, the spray programme - the time interval in which spraying is necessitated and the zones in space where chemical is to be sprayed, can be

scheduled. Thus selective spray scheduling is conceived essentially as a problem in transient spatial diffusion and consumption :

Accumulation rate = Diffusion rate - Consumption rate.

Diffusion is modelled using Fick's Law. Consumption, attributed to evaporation, conversion and other spray losses is assumed to be first order in spray concentration. Surface diffusion alone is considered because of lower density of spray chemical and to make the problem tractable. The surface concentration at the initial spray site is assumed invariant with time as usually it is a large value. The resulting partial differential equation has an analytical solution. Numerical solutions are needed if the concentration dependence of the consumption rate is other than one or zero. Incorporation of mosquito population dynamics into model lend scope for further improvements to the model. Analytical solution for various values of diffusivity and consumption rate constant are plotted and interpreted.

O - 7.17

RONALD ROSS AND ROBERT KOCH, COMPETITORS AND COLLEAGUES

Jan Peter Verhave
Lab. Medical Parasitology, University of Nijmegen, The Netherlands

Both pioneers on malaria transmission were in 1897 engaged in fieldwork : Ross in British India and Koch in German East Africa (Tanzania). Their common conviction was that mosquitoes were the vectors of plasmodia. Ross concentrated on his bird model, whilst Koch tried to gather circumstantial evidence from the field. Ross was a newcomer; Koch had enough credit of his own and was in fact already famous. Once back from Africa the newspapers praised Koch for revealing the role of mosquitoes, much to the annoyance of the British medical Journal which had Ross as the discoverer. Koch visited Italy in 1898 (Lombardy and the Pontine marshes), where he confirmed the existence of three plasmodial species and that pernicious malaria in Africa and Italy were caused by the same parasite. He showed that the flagellae were analogues of spermatozoa. Koch made also use of the bird model, confirmed the finding of Ross and succeeded to describe the sporogonic ookinete before Ross did. The Italian malariologists were quite uneasy and it speeded up their publication on anophelines transmitting human malaria (1898).

In 1899 Koch engaged in a scientific malaria expedition, which first halted in Italy again (Toscane) where he witnessed the summer-epidemic. Mosquitoes were then found infected, not in the cold season. They proceeded to Java in the Netherlands East Indies. Koch concluded that none of the doctors used the microscopical confirmation and thus, many fever cases were diagnosed incorrectly. He disputed the usefulness of sending patients to the hill resorts to recover from malaria. Quinine was the sole useful remedy. He concluded that malaria and its parasites were common in children; a survey among

adults would not give a proper indication of the prevalence of malaria. The expedition continued to German New Guinea (PNG) where he systematically treated patients with quinine. Intermittent prophylactic use at weekly intervals kept the people free of malaria. Furthermore, the theory of immunity to malaria in grown-ups was confirmed.

His reports were quickly published in the Deutsche medicinische Wochenschrift and he was praised as a champion of German science. Koch and Ross have kept irregular correspondence and it was Koch's advise to grant the Nobel Prize solely to Ross. Contrarily to Ross, the importance of Koch for malaria has gradually been forgotten.

O - 7.18

MALARIA IN THE PUNJAB : 1867 - 1943

Colin Butler
Dept. Epidemiology & Population Sciences, London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT, UK..

Modern India has, for the past two decades, experienced a resurgence of malaria including from *P. falciparum*. In the global context of increasing drug resistance by *P. falciparum*, insecticide resistance by vectors and climate change, malaria may worsen. The development of improved models to predict the occurrence of malaria therefore remains a major research priority. Re-analysis of historical data may help with this goal. The Punjab was subject to devastating epidemics of *P.falciparum* malaria between 1867 and 1943. A preliminary analysis for seven districts of the Punjab was made using data obtained from the India Office of the British Library as well as El Nino Southern Oscillation data from 1871. Explanatory variables analysed were climatic factors and irrigated acreage. Outcome variables were spleen counts for the whole province, from 1916 to 1943, the epidemic index and the incidence of annual fever mortality for each of the districts.

Early results confirm previously published findings that the severity of epidemic malaria declined between 1867 and 1943. No evidence was found, judging by spleen count records, to suggest this decline was because of transformation of malaria to a more endemic pattern,. There has been speculation that the expansion of the irrigation system in the Punjab may have increased malaria mortality, although an inverse relationship between irrigated acreage and the epidemic index occurs in this time. The epidemic prediction system, instituted from 1921 was based on parameters of population immunity, climate and food prices, taking no account of irrigation data. However, mortality from malaria may have been amplified by the building or presence of canals, in the early stages of expansion of the irrigation network. Between 1920 and 1941 rainfall was below average, but a contributing factor for the decline in malaria mortality in this period may have been improved treatment, in part responding to the warning system.

O - 7.19

S N Sharma
(Abstract not received)

O - 7.20

SERUM IRON STATUS OF PATIENTS WITH MALARIA

A K Kapoor[1], Nirmala[1], B Lal[1], G S Rao[1], A Kumar[2], G P Dutta[3] and P K Misra[2]
1. Division of Petroleum Toxicity, I T R C, Lucknow
2. Department of Pathology and Paediatrics, K G's Medical College, Lucknow
3. Division of Microbiology, C D R I, Lucknow

The various parameters of serum iron status have been analysed in a group of 30 *Plasmodium falciparum* patients, 30 MP negative fever and 30 healthy controls. Among the malaria patients. 27 cases were severely anaemic with mean packed cell volume (PCV) of 29.1±7.86 cc% ($p<0.001$) as compared to healthy controls (39.76±3.77 cc%). The level of PCV in MP negative fever cases was 32.73±5.28 cc% ($p<0.01$), which was also significantly lower when compared to the healthy controls. Malaria patients showed decreased mean serum iron (12.13±3.88 µml/l) but elevated serum total iron binding capacity (TIBC - 96.13±40.54 µmol/l and serum ferritin levels (136.25±109.9 µg/l) when compared with healthy controls (S. iron = 14.5±3.11 µmol/l:S. TIBC = 41.07±19.62 µmol/l;S. ferritin = 61.72±42.05 µg/l). MP negative fever cases also showed decreased mean serum iron (10.35±3.71 µmol/l), elevated serum TIBC (96.36±37.66 µmol/l) but serum ferritin (70.01±71.75 µg/l) was within normal range as compared to healthy controls. Elevated serum ferritin in malaria patients reflected adequate body iron stores. The present study suggests that iron supplementation may not be necessary for treatment of anaemia associated with malaria.

O - 7.21

MALARIAL ILLNESS AND TOLERANCE : TWO SIDES OF THE SAME COIN.

Ian A. Clark, Ian. G. Scragg* and Fadwa Al Yaman
Division of Biochemistry and Molecular Biology, Australian National University, Canberra, Australia, and * Department of Paediatrics, University of Oxford, John Radcliffe Hospital, Oxford, U.K.

Malarial illness and malaria tolerance provide good examples of the complexity involved in understanding the various roles nitric oxide can play in disease. As occurs with recurrent malarial infection, repeated exposure to bacterial endotoxin allows an individual to tolerate high doses without illness, a state referred to as endotoxin tolerance. Since human malarial infection generates tolerance to the harmful effects of both

malarial parasites and bacterial endotoxin, we have addressed the possibility that tolerance to each agent might, as with their acute pathology, share a common mechanism. Endotoxin tolerance is now appreciated to be an active process, with

O - 7.22

ANTIGENIC VARIATION IN *P. FLACIPARUM* : ECTOPIC RECOMBINATION INVOLVING SUBTELOMERIC *VAR* GENES

Scherf, A, Bottius E , Hernandez-Rivas, R , [1]Pouvelle, B , [1]Gysin J , [2]Lanzer, M , [3]Wellems, T E
Institut Pasteur, Paris;
[1]Institut Pasteur, Lyon, France;
[2]Universitat Wurzburg, Germany;
[3]NIH, Bethesda, USA.

Antigenic variation and cytoadherence of *Plasmodium falciparum* infected erythrocytes are modulated by a family of variant surface proteins encoded by the multigene *var* family. var genes are dispersed on most if not all chromosomes and 50 to 150 genes are estimated to be present in the haploid genome. Transcripts from *var* genes have been mapped to central as well as subtelomeric regions approximately 20 to 40 kb from the telomere repeats. DNA hybridization analysis revealed that *var* genes are not commonly shared by genetically different parasite strains. In some cases, comparisons of individual isolates have shown identical or very closely related single-copy *var* genes in the subtelomeric regions of different chromosomes, suggesting that the *var* genes undergo transposition events and are therefore mobile.

We analysed the significance of the observed *var* mobility with regard to the mechanism(s) that control switches of *var* gene expression and the diversity of the *var* gene repertoire in genetically different parasite strains. Parasite subpopulations with different cytoadherent phenotypes were selected from a cloned laboratory parasite line. PFG and fine mapping analysis of those parasites did not reveal any repositioning of the activated *var* genes. In switch events, *in situ* activation, possibly involving trans-activating factors, may play an important role in *var* gene regulation. However, after meiosis, we observe high frequencies of DNA rearrangements involving *var* genes. In nearly half of the progeny clones from two crosses of *P. falciparum*, recombination events between *var* genes on different chromose ends lead to the transposition of *var* sequence from one chromosome to the other, or to the presence of a duplicated copy of the *var* sequence on a different chromosome (ectopic recombination). Our finding suggests that ectopic recombination plays a crucial role in the generation of *var* gene repertoire diversity.

O - 8.1

HAEMOSPORIDIA OF BIRDS OF NORTH COASTAL ANDHRA PRADESH, INDIA.

C Kalavati[2] and M Sambasiva Rao[1]
1. Chief Minister's Secretariat, Government of Andhra Pradesh, Hyderabad
2. Division of Protozoology, Department of Zoology, Andhra University, Visakhapatnam-530 003.

Blood films examined from 395 birds represented by 25 genera and 19 families collected from 3 districts of north coastal Andhra Pradesh (Visakhapatnam, Vizianagaram and Srikakulam) together comprising the north physiographic province of eastern ghats, during 1982 -'83, revealed altogether 10 new species of haemosporidians - 4 of *Plasmodium* represented by one each of the sub - genera *Haemaoeba* and *Novyella* and 2 of *Giovannolaia*, 4, *Haemoproteus* and 2 *Leucocytozoon*. Overall, 16.2% of the birds examined harboured either single or multiple infections of *Haemoproteus* (10.6%), *Plasmodium* (3.1%) and *Leucocytozoon* (1.8%). Prevalence of infection was maximum in the common mynah, *Acridotheres tristes*, (25.0%), followed by the Jungle babler *Turdoides striatus* (15.6%), the Koel *Eudynamus honarata* (9.37%) and the house sparrow *Passer domesticus* (9.37%). Prevalence was low in the central arid zones (12.19%) than in birds collected from the plains of north eastern region (27.13%) or high altitudes of west and north western parts (60.67%). Intensity was in general moderate. The paper describes in detail the morphological characters of the parasites encountered together with information on taxonomic identification and distribution of the species.

O - 8.2

SENGA STRIATUSAE NEW SPECIES FROM A FRESH WATER FISH

Chalobol Wongsawad[1] and B V Jadhav[2]
1. Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
2. Department of Zoology, Dr. B.A. Marathwada University, Aurangabad, India.

The present communication deals with a new species of the *Senga* Dollfus, 1935 as *S. striatusae* from a fresh water fish *Channa striatus*. The present form differs from all the known species of the genus with pear shaped scolex, 17-19 hooks, presence of neck, mature segments broader than long, testes 85-90 in number, oval cirrus pouch, elongated ovary, vagina anterior to cirrus pouch and follicular vitellaria.

O - 8.3

ON SOME HAEMOSPORIDIAN GENERA OF FISHES OF VISAKHAPATNAM

K Padma Dorothy and C Kalavati
Division of Protozoology, Department of Zoology Andhra University, Visakhapatnam 530 003.

Studies on haemosporidians of piscean hosts are a few and fragmentary. A three year study (1985-'88) on haemosporidia of fishes of Visakhapatnam revealed the presence of 4 species belonging to the genera : *Mesnilium, Babesiosoma* and *Haemogregarina.* Among them the most significant is the occurrence of a species of *Mesnilium* infecting the mullets, *Mugil cephalus* and *Liza macrolepis* found in the polluted backwaters of Visakhapatnam. Erythrocytic schizogony resembles closely that of the genus *Plasmodium.* Exoerythrocytic schizogony seen in liver. Prevalence of infection low (2.0%). Vector not known. Two species of *Babesiosoma* and each from the mudskipper, *Channa* sp. and the catfish *Arius* sp. inhabiting the freshwater ponds near Visakhapatnam were described. *Haemogregarina* sp. is encountered in the blood of the marine fish, *Echinis carinata* collected from the offshore landings at Visakhapatnam. The paper describes in detail the erythrocytic stages and their systematic position.

O - 8.4

STUDIES ON LARVAL CESTODES OF MARINE TELEOST FISH PARACHAETURICHTHYS POLYNEMA

C Vijaya Lakshmi and M Damodara Rao
Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Though there is ample information on the complete life cycles of some cyclophyllidean and pseudophyllidean cestodes, knowledge concerning the life cycles of many other groups of cestodes is incomplete. There are reports of some of the larval stages of these orders from a variety of hosts like copepods, ctenophores, decapod crustaceans, molluscs and teleost fishes. The lacuna in the knowledge in this field may be because of the occurrence of these groups of cestodes in deep sea fish like elasmobranchs and also because of difficulty in maintaining the adult and larval cestodes in the laboratories.

In the present study of cestode larvae of Waltair coast, the marine teleost fish *Paracheaturichthys polynema* is found to harbour a variety of cestode larval species belonging to the different orders. Ten different larval species belong to the order Tetraphyllidea and one larval form belonging to Trypanorhyncha have been collected. These larvae have been fixed for taxonomic studies and identified up to the genus level and their position has been discussed.

O - 8.5

A NEW SPECIES OF *LAIMYDORUS* SIDDIQI, 1969 FROM THE RHIZOSPHERE OF *PHASEOLUS VULGARIS* IN ROHILKHAND DIVISION, UTTAR PRADESH (INDIA)

Sunita Sharma and Mahjabeen Khatoon
Pest and Parasite Research Laboratory, Department of Zoology, Bareilly College, Bareilly (U.P.)

A new species of Laimydorus (Nematoda : Dorylaimoidea, De Man 1876) has been isolated from the soil, found around the roots of *Phaseolus vulgaris* (Bakla) in Rohilkhand Division, U.P. The female is 1.5mm long and it has marked lip region. Odontostyle is 32 µm long and the odontophore is 28 µm in length. The nerve ring is present at a distance of 146.7 µm from the anterior end. The guiding ring is present at a distance of 24 µm from the anterior end. Prerectum and rectum measure 44 µm and 24 µm in length, respectively. Cardia is tongue shaped and it is 16 µm long. In *Laimydorus partapurii* n.sp. a = 30.9, b=4.5, c=20.9, c' = 2.3 and v=52%. The males of this species were not found. The new species is named as *Laimydorus partapurii.*

O - 8.6

TWO NEW SPECIES OF SOIL NEMATODES OF GENUS *PORONEMELLA* SIDDIQI, 1969 (DORYLAIMOIDEA : LORDELLONEMATINAE) FROM ROHILKHAND DIVISION, UTTAR PRADESH

Mahjabeen Khatoon and Sunita Sharma
Pest and Parasite Research Laboratory, Department of Zoology, Bareilly College, Bareilly - 243005 (India)

Two new species of the genus *Poronemella* viz., *Poronemella vulgarii* n.sp. and *Poronemella_thiraii* n.sp, collected from the soil found around the roots of *Phaseolus vulgaris* (Bakla) and *Phaseolus lunatus* (Lobhiya) have been described from Bareilly (Rohilkhand Division, U.P.). *Poronemella vulgarii* n.sp. is 1.3mm long with a = 27.3, b = 4.8, c = 33.4, c' = 1.25, v = 51%, odontostyle = 24 µm, odontophore = 40 µm, oesophageal length = 228.2µm, cardia = 16µm, G_1 = 163µm, G_2 = 200 µm, NR = 130µm, Prerectum = 28µm, Rectum = 16µm, and is closely related to *Poronemella amini.* It however, differs in having shorter body size (1.3 *vs* 1.7) mm, longer spear (60µm *vs* 20 µm) and the values of a (27.3 *vs* 43), b (4.8 *vs* 4.2) and c (33.4 *vs* 15). *Poronemella thiraii* n.sp. is characterised by l = 1.1 mm, a = 29.7, b = 3.8, c = 33.0, c' = 1.8, v = 52%, Tail 36µ, odontostyle = 28µm, odontophore = 44µm, esophageal length = 268.8 µm, cardia = 16µm, G_1 = 164µm, G_2 = 179 µm, NR = 146 µm, prerectum = 81µm, Rectum = 32 µm and is close to *Poronemella porifer.* It may however, be distinguished from this species in values of a (29.7 *vs* 32), c (33.4 *vs* 14) length of odontostyle (24µm *vs* 17µm) and size of tail (26µm *vs* 74µm).

O - 8.7

CARYOPHYLLIDEAN CESTODE PARASITES OF FRESH WATER FISH IN KASHMIR

Fayaz Ahmed and M A Chishti
Post - Graduate Department of Zoology The University of Kashmir, Srinagar - 190 006, Kashmir.

There has been a great controversy about the status of the family Caryophyllaeidae Leuckart, 1878. Caryophylli-deans being unique in having a monopleriod body with a single set of reproductive organs in a non segmented body have invited speculation concerning their systematic position. Central to such speculation have been the role of progenesis in evolution and the question whether the monozoic body plan represents a primitive condition or arose secondarily, so a brief review has been attempted. Fish hosts belonging to different fish species; collected from Dal, Anchar, Manasbal and Wular lakes and river Jhelum; dissected in 0.7% NaCl and cestodes recovered. Live/ fresh parasites were killed in hot water (65°C), fixed in Carnoy's fluid under slide pressure, stained regressively in Bullough's Aceto-Alum-Carmine, and mounted in D.P.X. Microanatomy was conducted under Olympus Research microscope and micromeasurements with micrometers and expressed in mm. In addition to the genotype *Adenoscolex oreini* Fotedar, 1958 from the intestine of *Schizothorax niger* and *S. esocinus* from river Jhelum, Dal and Wular Lakes, *Adenoscolex fotedari* sp. novo. from small intestine of *Schizothorax niger* from Anchar and Manasbal lakes has been described. In the former 10 hosts were found to be infected with 394 cestode specimens, whereas in the latter case 5 infected fish hosts were found to harbour 269 specimens. Fresh specific diagnosis has been given for the species. Further comparison of genera of the subfamily Capingentinae Hunter, 1930 has been given in a tabulated form.

O - 8.8

ON THE SPECIFIC DIAGNOSIS OF JUVENILE *PARAMPHISTOMUM EPICLITUM*

A Prasad and T K Varma.
Division of parasitology, India Veterinary Research Institute, Izatnagar - 243 122.

Juvenile/immature paramphistomes were collected from small intestine of experimentally infected lambs and kids given 3500 metacercariae *per os.* Metacercariae of *P. epiclitum* were obtained after exposing *Indoplanorbis exustus* snails to miracidia of *P.epiclitum.* Flukes at 2, 4, 8, 12, 16,and 20 weeks - were studied morphologically and histologically. Flukes at 2, 4 and 8 weeks showed 7 bends in the intestinal caeca ending behind acetabulum, pharynx showed small dome shaped papillae and the terminal genitalium was of "epiclitum" type in the immature flukes. Uterus was median and genital pore was always behind the intestinal bifurcation irrespective of age of fluke and method of fixation. Crossing of Laurer's canal of excretory bladder could be observed

at 12 weeks PI only when reproductive organs became fully developed. In 16 weeks old flukes in kids and 20 weeks in lambs median tandem lobed testes, post testicular ovary and median uterus were present. But the most prominent feature was development of sucker like terminal genitalium which contained only few radial fibres and was not a typical genital sucker. Crossing of Lauter's canal and excretory bladder could be observed.

It was concluded that for specific diagnosis of juvenile/immature flukes from intestine certain characters e.g. form of intestinal caeca, position of genital pore, absence of lip splinter in pharynx "epiclitum" type of terminal geniitalium and presence of dome shaped papillae in the pharyngeal cavity are much helpful. This study may be very much useful in differentiating *P. epiclitum* from *P. cervi* and other related species.

O - 8.9

HISTOPATHOLOGICAL STUDIES ON A CARYOPHYLLIDEAN CESTODE GENUS - *ADENOSCOLEX* INFECTING FRESHWATER FISH IN KASHMIR

M Z Chishti and Fayaz Ahmed
P.G. Department of Zoology, University of Kashmir, Srinagar-6

When the host-parasite equilibrium is disturbed, serious disease or epizootics in the host is the consequence, resulting in the depletion or weakening of the host. Cestodes are well known to produce certain adverse effects on their piscine hosts. These include mechanical blockage of the gut lumen, production of lesions and in the physiological state of the host thereby predisposing it to other infections. The present endeavour, to work out the pathology of *Adenoscolex* Fotedar, 1958 infecting *Schizothorax* spp. was undertaken as this important aspect has been totally neglected in this region. Fish hosts from different water bodies in Kashmir were dissected in 0.7% NaCl for cestode parasites. Any obvious damage was recorded and the infected and uninfected fish intestine were fixed in aqueous Bouin's fixative, infiltrated with paraffin wax, sectioned at 5-20 microns, sections affixed with Mayer's affixative and permanent preparations were made using Mallory's triple stain. *Adenoscolex* was always found free in the intestinal lumen and never attached. In light infection *Adenoscolex* was found predominantly in the duodenal region of the intestine whereas in heavy infection the lower intestine was also found to be infected. In the latter case the infected region was distended and transparent and sometimes resulted in rupture of intestine and mechanical obstruction of gut, however, no nodule formation was observed. Histopathological studies revealed complete disruption of mucosal layer which would extend to submucosa or even to muscularis, further the intestinal villi or the mucosal folds got compressed due to the parasite presence. Thus *Adenoscolex* in the absence of any holdfast organs was found free in the lumen causing pathological changes in the form of shortening of intestinal villi or compression of the mucosal folds as a consequence of pressure necrosis, mechanical obstruction, lesion production and general physiological imbalance in *Schizothorax*.

O - 9.1

C Ghish
(Abstract not received)

O - 9.2

FIELD TRIAL WITH LAMBDA-CYHALOTHRIN AS AN EFFECTIVE AND SAFE INSECTICIDE AS RESIDUAL SPRAY FOR CONTROL OF MALARIA VECTORS

P K Srivastava[1], K Ravi kumar[1], B R Thapar[2], R D Joshi[2], R L Yadava[3], C Krishna Rao[3], A Prakash[4], and Shiv lal[2]
1. Regional Office of Health and Family Welfare, Bangalore
2. Directorate of National Malaria Eradication Programme, 22-Sham Nath Marg, Delhi - 110 054
3. Former Officials of Dte. NMEP, Delhi.
4. Dte. of Health Services, Karnataka state, Ananda Rao circle, Bangalore.

A three year field trial with lambda-cyhalothrin 10 WP conducted in triple resistant vector areas of Kolar and Tumkur districts of Karnataka state from 1991 to 1993 through Primary Health Care System, adopting dosages of 15 mg/m^2 (for three cycles of spray annually) and 30 mg/m^2 (for two cycles of spray annually) gave promising results in reducing the density of malaria vector, *Anopheles culicifacies* and transmission of malaria in the community.

Lambda-cyhalothrin spray brought marked reduction in the total malaria cases and Pf cases by 79% and 95% in the second year and 53% & 50% in the third year respectively after spray where 30 mg/m^2 dose was adopted. The lesser impact in the third year could be probably due to migration of nomadic population. In the comparison area the malaria cases and Pf cases increased by 334% and 285% respectively during 1993 as compared to 1991.

In areas sprayed with 15mg/m^2 dose the decline was 67.0% and 92% respectively in total malaria cases and Pf incidence after three years of spray.

In 30 mg/m^2 dose trial area density of vector mosquito declined to zero after lambda-cyhalothrin spray and remained suppressed throughout the trial period, whereas in comparison area sprayed with DDT (1 g/m^2 two cycles a year) the vector mosquito density increased by twice and thrice. Similarly in 15 mg/m^2. area the vector mosquito density was suppressed to zero after lambda-cyhalothrin spray and remained low throughout the trial period whereas in comparison area mosquito density showed increase.

Monitoring of toxicological effect of lambda-cyhalothrin on human health including systematic investigation and nerve conduction test indicated no untoward ill effect on

human subjects exposed to this synthetic pyrethroid.

The observations indicated that in areas sprayed with lambda-cyhalothrin household pests like cockroaches, house- flies, headlice,lizards, etc. were eliminated thereby conferring collateral benefit to the community in getting rid off these pests which facilitated good spray coverage due to community cooperation.

O - 9.3

B R Thapar
(Abstarct not received)

O - 9.4

MALARIA TRANSMISSION, VECTOR CONTROL AND PERSONAL PROTECTION

Umberto D' Alessandro
Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium.

Recent studies on the impact of insecticide-treated bednets (ITN) have shown a considerable impact on childhood mortality ranging from 17% to 33%. However, concern on long-term efficacy of an ITN programme on child mortality has been raised by several authors. These are based on the observation that variations of malaria-specific mortality and severe disease are weak compared to the considerable range of transmission levels and that only a considerable reduction of transmission will have a long-term effect on the burden of malaria. A careful examination of the evidence reveals important problems. Data from Brazzaville indeed show that extreme differences in malaria transmission may be associated with only minor differences in severe malaria incidence rates. However, it should be noted that these were much lower than those reported from other African regions, the widespread use of antimalarials and the higher socioeconomic status have been suggested as possible reasons, and are clearly unrepresentative of most of sub-Saharan Africa. Data on the relation between malaria mortality rates and intensity of transmission were reviewed recently by Snow and Marsh. Malaria mortality rates were determined by using verbal autopsies and the unreliability of this approach has been stressed already. In some cases, information on the causes of death was collected several years after the event, making the diagnosis even more unreliable. Another factor of unreliability is the use of different methods to estimate the intensity of transmission. There is the urgent need to establish the relation between intensity of transmission and malaria mortality and particularly to evaluate the long term effects of ITN. The evidence accumulated so far, is not sufficient to warrant abandoning the implementation of ITN programmes.

O - 9.5

OPPORTUNISTIC AND NON-OPPORTUNISTIC INTESTINAL PARASITIC DISEASES IN HIV POSITIVE PATIENTS IN MANIPUR - THEIR PREVALENCE AND EPIDEMIOLOGIC CHARACTERISTICS

Laitonjam Anand and Ch Dhanachand
Parasitology Laboratory, Department of Life Sciences, Manipur University, Canchipur 795 003.

A survey on the intestinal parasitic diseases among the HIV positive asymptomatic IVDUS (intravenous drug users) of Manipur revealed the presence of 3 protozoans and 2 nematodes. Of these, the two opportunistic parasitic protozoans i.e. *Cryptosporidium* sp. and *Isospora* sp. were predominant over *Entamoeba histolytica*, While non-opportunistic parasitic nematodes i.e. *Ascaris lumbricoides* and Hookworm were the less detected ones. In all cases, bacterial flora were predominant over the fungal flora and the fungus *Candida* sp., was the predominant one compared to others like-*Cladosporium* and *Humicola*.

The study also reveals that although these individuals show no gastrointestinal disorders like diarrhoea, etc. they still harbour these opportunistic and non-opportunistic parasites and act as carriers, particularly of these opportunistic ones in the community and they are responsible for disseminating these opportunistic parasites via contaminated water through open defaecation system. This poses a threat to the health of the community, since these opportunistic parasites can colonize in the GI tract, whenever they encounter an immunologically weak and incompetent individual. Since HIV positive persons are maximum in the state, unless a precautionary measure is taken up with an immediate effect, all those immunologically incompetent / compromised individuals, irrespective of whether HIV positive or negative, may be suffering from diarrhoeal diseases due to these opportunistic pathogens in the future.

O - 9.6

COMMUNITY BASED INTEGRATED MANAGEMENT OF *AEDES AEGYPTI* IN DELHI NCR

M A Ansari
Malaria Research Centre, 22, Sham Nath Marg, Delhi-110 054.

Aedes aegypti is a principal vector of Dengue and Dengue haemorrhagic fever (DHF) in peri urban and urban area's of Delhi National Capital Region (NCR). It breeds in peridomestic and domestic containers. Potential breeding places are tyre dumps, scrap materials, coolers, overhead tanks, ornamental pots, etc. There is no organized- national control programme for Dengue and DHF in the country. Municipalities /corporations are entrusted to carryout antilarval measures occasionally supplemented with ULV fogging and residual spray in and around premises of positive case of falciparum ma-

laria under Urban Malaria Scheme (UMS). The strategy did not provide any dent in either preventing focal malaria outbreaks or dengue and DHF. Recent outbreak of the disease in 1996 in Delhi affected >8000 people and caused 400 deaths. Earlier surveys carried out in Delhi revealed that domestic breeding places mainly act as mother foci of *Aedes aegypti* in Delhi. With the onset of rains, breeding spreads to peridomestic containers. Community based source reduction *viz* weekly de-watering and proper covering of clay pots, overhead tanks receptacles, etc. and solid waste management can provide effective control of the *Aedes aegypti* breeding. Community can also supplement control by taking personal protection measures such as repellents, curtains, aerosols, etc. Legislative measures and model building bylaws may be enforced to eliminate mother foci particularly during the non-transmission period. Results of surveys and control of *Aedes aegypti* would be discussed.

O - 9.7

V D Broel
(Abstract not received)

O - 9.8

ROLE OF NON-GOVERNMENT ORGANIZATIONS AND COMMUNITY PARTICIPATION IN MALARIA CONTROL

P N Sehgal
Voluntary Health Association of India, 40 Institutional Area, New Delhi - 110 016.

Inadequate and poorly developed peripheral health care services and lack of effective community involvement are among the important factors for resurgence of malaria in India. Non-Government Orgainsation (NGOs) i.e. voluntary agencies and provate practitioners working closely eith the community can fill up this void with great degree of success. A rlevant and operational linked between government and NGOs in malaria control should be evolved to involve them not only as alternative service providers but also as health communicators, community mobilizers, alternative community health oriented trainers, awareness builders for community participation, action and operational researchers. The voluntary agencies can play significant role in early diagnosis and prompt treatment of malaria cases, health education for personal protection from mosquito bites and integrated mosquito control measures.

Voluntary agencies with commitment to the malaria programme may be identified and devoloped as Resource centres. Thewy should lbe given proper training and material support for lsffective partnership in the implementation of the programme. The cost of the training community based NGOs should be incorporated into the regular budget of National Malaria Eradication Programme.

Private practitioners should be educated and oriented through Continuing Medical Education programme with the help of professional organizations and communication material on rational malaria diagonosis and treatment regimens.

The community should be actively involved in planning and implementation of maleria control lstrategies as per local needs. They should be prepared as participants rather than passive beneficiaries of the programme. The strategy should be directed towards enabling and empowering the community for prevention and control of malaria. Community based NGOs can facilitate community participation through awareness campaigns. Appropriate strategies for the involvement of NGOs and community participation are highlighted in the paper.

O - 9.9

'KAP' IN RELATION TO MALARIA AND ITS CONTROL IN AN URBAN AREA

G Naravanasamy[1], C Rajamanickam[1], N C Appavoo[1] and R Selvaraj Pandian[2]
1. Department of Public Health and Preventive Medicine, Chennai 6
2. The American College, Madurai-625 020.

As a part of an effort to understand the community involvement to contain persistent malaria transmission and its control, we studied the knowledge, attitude and practices (KAP) of residents of Dindigul town in Tamil Nadu. Most residents are aware of the malaria episode and recognise the role of mosquitoes in malaria transmission, but few about the type of mosquito which is responsible for the transmission. More than 80% accept blood test as method to diagnose malaria. Only about 60% are aware of the malaria clinics that function for that purpose. The risk of having untreated person in their midst is not known to them. The fact that Anopheline mosquitoes breed in fresh water collections is little known even to the educated residents. Though the antilarval work is being carried out for over 2 decades, only about 43% has understood that Municipality is responsible for this activity. Less than 5% of the families use bednets, but the use of mosquito repellents especially mats and coils is found to about 56%. Even though malaria is a notifiable disease in Tamil Nadu, less than 1% know about the fact. The residents have a very positive opinion of the N.M.E.P., since they believe that the malaria problem is taken care by the programme personnel and very little regard has been shown to Municipal personnel because of their negligence is carrying out the mosquito control activities. The prevalence of malaria is known by the community mainly through mass media like T.V., Radio and News papers. The residents are not aware of the usefulness of larvivorous fishes, proper screening of the water storage facilities and source reduction methods to control mosquito breeding. Adequate facilities for screening the fever cases, prompt treatment through the available health and medical facilities are the felt need of the residents, since the knowledge, beliefs and practices of the urban residents vary from one locality to another.

O - 9.10

RESIDUAL EFFICACY OF DELTAMETHRIN & CYFLUTHRIN IN DISTRICTS OF KARNATAKA STATE, INDIA.

K Ravi Kumar[1], P K Srivastava[2], C V Ponnappan[1] and Shiv Lal[3].

1. Regional Office for Health and Family Welfare, Bangalore, Karnataka.
2. Regional Office for Health and Family Welfare, Chennai, Tamilnadu.
3. National Malaria Eradiction Programme, Delhi.

Udayapura Primary Health Centre of Hassan district was under spray with Deltamethrin 2.5 %w.d.p. @ 20 mg/m^2 and Turuvekere Primary Health Centre of Tumkur district was sprayed with Cyfluthrin 10% @ 25mg/m^2 in the year 1996. To study the residual efficacy of both these insecticides on sprayed wooden / cement plastered walls, Cone-Bioassay Tests were carried out using *Anopheles culicifacies* as test insect, which is a known vector in these areas. The study revealed that, in case of Deltamethrin, 100% mortality was recorded upto 36 weeks on wooden surface, which declined to 80% in case of cement plastered walls, 36 weeks after spray. In case of Cyfluthrin, 100% mortality was recorded from both wooden and cemented surfaces upto 15th week which declined to 90% after 16th week and remained constant upto 36th week on wooden surface. On cement plastered surface the mortality was reduced to 40% on 17th week and the mortality remained same upto 36th week. The longer residual efficacy of both these Pyrethroid insecticides showed their promising role on residual insecticide in malaria control.

O - 10.1

ANTIBODIES AGAINST DIFFERENT DOMAINS OF P0, A RIBOSOMAL PHOSPHOPROTEIN, INHIBIT *PLASMODIUM FALCIPARUM* GROWTH *IN VITRO.*

Sanchita Chatterjee, Subhash Singh and Shobhona Sharma.
Molecular Biology Unit, Tata Institue of Fundamental Research, Homi Bhabha Road, Bombay, India.

A differential immunoscreen using immune and patient sera detected antibodies against amino-terminal of P0, a ribosomal phosphoprotein homologue of *Plasmodium falciparum,* exclusively in the malaria immune persons. Different domains of P0 spanning the whole protein have been expressed as GST fusion proteins in *E. coli.* Polyclonal antibodies have been raised against these domains and they do recognize the 38kDa P0 protein from parasite extract on Western blot. About 10-15% patients suffering from Systemic Lupus erythrematosus (SLE) an autoimmune disease also possess antibodies against P0. Such SLE patients sera, having high titre anti-P0 antibodies, recognize only the carboxy-terminal domain but not the aminoterminal domain. Polyclonal sera against both the aminoterminal and the carboxy-terminal domains of P0, and also the SLE patients sera inhibit parasite growth *in vitro.*

O - 10.2

VACCINATION WITH MYCOBACTERIUM HABANA ALONGWITH KILLED LEISHMANIA PROMASTIGOTES AGAINST L. DONVANI INFECTION IN LANGURS.

*H P Gupta**, P. Sharma, J K Srivastava, A Misra, Anuradha and J C Katiyar
* Division of Microbiology and Division of Parasitology, Central Drug Research Institute, Lucknow-226001, India.

Recent trends in the control of leishmaniasis melady has been with the use of polyvalent vaccine(s). A combination of killed promastigotes alongwith BCG have been used aginst cutaneous leishmaniasis with success. BCG presumably has has imparted adjuvant action with promastigotes to enhance immunity. With this view point, another immunogenic strain of Mycobacterium designated M.habana (M.simiae Seovar-1) a candidate vaccine for leprosy when evaluated alongwith killed promastigotes accorded 65-70% prophylactic action against the challenge ection of L.donovani in hamsters. This promoted us to assess its efficacy in langurs. A group of 4 monkeys were vaated with killed L.donovani promastigotes + M. habana vaccine and another group of 4 monkeys was kept as unvaccinated control. The vaccinated control monkeys were challenged with 1 x 10^8 amastigotes i.v. and parasitic assessment in splenic tissue was done at different intervals. The results reveal that this combination has exerted 50% protection in langurs. Future prospects of this combination will be discussed.

O - 10.3

IMMUNOPATHOLOGY AND IMMUNOMODULATION OF TPE

Rajeev K Malhotra, Eric Pearlman and James W Kazura
Division of Geographic Medicine, SOM, Case Western Reserve University, Cleveland, OH, USA

Tropical Pulmonary Eosinophilia (TPE) is a severe form of allergic asthma, though to be caused by an inflammatory response to filarial larvae (Mf) trapped in lung microvasculature. The characteristic features of this disease, such as eosinophilia, marked increases of filarial-specific IgG and IgE, and airway hyperreactivity associated with the presence of eosinophilis in lung, can be reproduced in sensitized mice by i.v. inoculation of Mf. These animals developed a pronounced Th2 response in spleen and luwith increased productionof IL-4 and IL-5 in comparison to that of IFN-ν. Serum IgE and blood eosinophils levated, and eosinophils comprised up to 56% of total cells in BAL fluid. Eosinopohil MBP was present on respory epithelial cells, and animals developed airway hyperresponsiveness, as measured by tracheal smooth muscle ronse to exogenous Ach. To determine if these Th2-associated responses could be modulated, animals were given I2 at the time of initial sensitization with parasite larvae. IL-12 skewed the response in spleen and lungs to Th1 like, with elevated IFN-ν and decreased IL-4 and IL-5 production. Consistent with this shift in cytokine response, antigen-specific IgG2a was elevated and IgG1 and total serum IgE wer decreased. Eosinophils in blood and lung were reduced, and there was no detectable airway hyperreactivity compared to that in saline injected control animals. These data indicate that helminth-induced airway hypersensitivity is asociated with the presence of eosinophils and release of eosinophil MBP into airways, and suggest tha IL-12 may be of potential use in immunotherapy of this and other asthmatic diseases.

O - 10.4

CHARACTERISATION OF PUTATIVE PROTECTIVE ANTIGENS OF *PLASMODIUM FALCIPARUM*

Mrinal Kanti Bhattacharyya, Charusheela Deshpande and Shobhona Sharma
Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha road, Bombay, India.

We have earlier reported *Plasmodium falciparum* epitopes against which antibodies are present only in the clinically immune individuals, but not in the acute malaria patients. We postulate that these epitopes may represent conserved and less immunogenic protein domains, immune response against which may be generated only after repeated exposures. One of these epitopes belong to P0, a conserved functional phosphoriboprotein. All the others represent novel cDNA clones. Our PCR results show that three of these epitopes are present in all the strains of *P. falciparum* tested and gene homologues of these were found to be amplified by PCR in *P. berghei,* a rodent ma-

larial parasite, and *P. vivax,* a differnt species of human malarial parasite. From the predicted open reading frame of one of these clones, Pf2, an oligo peptide has been synthesised and conjugated with BSA. Antibodies have been raised against this conjugated protein in rabbit. The anti peptide antibody picked up a 95 kDa parasite protein on Western blot. The IFA data shows the presence of expressed protein in blood stages of the parasite. The same antibody partially inhibits the growth of *P. falciparum* in *in vitro* culture. Further characterisation of these clones will be presented.

O - 10.5

HUMAN T AND B CELL RESPONSES OF *PLASMODIUM FALCIPARUM* MSP-2 ANTIGEN IN A HOLOENDEMIC AREA OF WEST AFRICA : DIELMO VILLAGE (SENEGAL)

P Dubois[1], J Iqbal[1], F Ntoumi[1], A Dieye[2], C Rogier[2], J F Trape[3], O Mercereau-Puijalon[1], C M Rzepczyk[4] and J L Sarthou[2].

1. Pasteur Institute of Paris, France.
2. Pasteur Institute of Dakar, Senegal.
3. ORSTOM, Dakar, Senegal.
4. Queensland Institute of Medical Research, Australia

The village of Dielmo, Senegal has been followed since six years in an epidemiological study, in order to define acquired resistance to *P. falciparum* malaria in terms of clinical premunition as well as ti parasite immunity- This study allowed the comparison of responses to the vaccine candidate merozoite surface antigen MSP-2, on the basis of acquisition of resistance to malaria in natural conditions in a hyperendemic area. At the MSP-2 locus, two allelic families can be defined by semi conserved sequences, and a central variable region is responsible for a very high polymorphism.

In a cohort of villagers, subclass analysis of antibody responses showed a large dominance of cytophilic IgGs among antibodies to MSP-2. However, our results suggest a very fast progression of MSP-2- specific cytophilic antibodies in the very first year of life long before the acquisition of clinical protection. Antibody responses against the two allelic families appeared strongly correlated, but only for cytophilic IgG responses, as IgG2 and IgG4 responses were different depending on the allelic family. Peptides containing different T-cell epitopes seemed to induce different cellular responses, and a TH0-like cytokine pattern was observed in children against an epitope common to both allelic families.

These differences between responses to different epitopes on the same antigen suggest that regulation of the antibody responses may vary depending on the allele encountered by the immune system during a new infection, different cytokine patterns eliciting different help to the B cell compartment.

O - 10.6

ANTI-rK39 ANTIBODY LEVELS IN ASYMPTOMATIC FAMILY MEMBERS OF INDIAN KALA-AZAR PATIENTS

S Singh[1], R Singh[1], R K Das[2], J Kumar[1], K P Chang[3] and S G Reed[4]
1. All India Institute of Medical Sciences, New Delhi,
2. NICD, Patna (India)
3. Chicago Medical School, Chicago
4. Infectious Disease Research Institute, Seattle (USA)

INTRODUCTION : Visceral Leishmaniasis in India is caused by *Leishmaina donovani.* However, it is not yet established in India that whether all the infected people develop kala-azar or some of them will remain asymptomatic.

AIMS & METHODOLOGY : To study this possible carrierness, we carried out a field study in kala-azar endemic areas of Samastipur (Bihar) in which anti-leishmanial antibody titres were determined in asymptomatic family members of the parasitology proven index kala-azar cases cases using a 39 aa long recombinant antigen (rK39) in microwell ELISA. All family members were followed up for 6 months.

RESULTS : A total of 54 family members from 6 households with 8 index cases were tested. Of these 12 (22.2%) were found to be positive for anti-rK39 antibodies. Of the 12 antibody positive cases, 5 developed clinical disease within 3 mo and all within 6 mo of the first testing. Two patients died of kala-azar.

CONCLUSION : This study indicates that using a highly sensitive and specific antibody detection assay like rK39 ELISA, sub-clinical infections with *Leishmania donovani* can be diagnosed and the results can be exploited for future vaccination programmes.

O - 10.7

EXPRESSION OF THE APICAL MEMBRANE ANTIGEN-1 (PcyAMA-1) OF *PLASMODIUM CYNOMOLGI BASTIANELLI*

Sheetij Dutta[1], Roop S. Bora[2], V.S. Duttu[2], Pawan Malhotra[2], Parimal Das[3], Clemens Kocken[4], O.P. Shukla[1], Sunil Lal[2], S.E. Hasnain[3], Alan Thomas [4] and V.S. Chauhan[2]
1. Central Drug Research Institute, Lucknow, India.
2. International Centre for Genetic Engineering and Biotechnology, New Delhi, India.
3. National Institute of Immunology, New Delhi, India.
4. Biomedical Primate Research Centre, Rijswijk, The Netherlands.

AMA-1 is one of the most promising asexual blood stage vaccine candidate antigen. *P. cynomolgi* B is human transmissible simian parasite and closely resembles the human malaria parasite *P. vivax.* To evaluate the vaccine potential of PcyAMA-1 in the experi-

mental models we have cloned and sequenced the PcyAMA-1 gene. The antigen was expressed both in prokaryotic and eukaryotic systems. The bacterial recombinant protein has been purified using Ni affinity column and refolded; immunological characterization of this antigen is underway. Among the eukaryotic systems the antigen has been successfully expressed in baculovirus and yeast expression systems. The recombinant antigen from baculo-virus has been partially purified although the yields are moderate. Among the yeast expression systems, the gene was expressed both in *Pichia* and *Saccharomyces*. The yield of secreted antigen from *Pichia* is low, however *Saccharomyces* system appears to be giving the best results among the eukaryotic expression systems. The *Saccharomyces* clone is being grown in a fermentor to produce large amounts of the antigen. The antigen has been purified and is awaiting immuological characterization.

O - 10.8

IN VITRO INHIBITION OF *SETARIA CERVI* SUPEROXIDEDISMUTASE BY HUMAN FILARIAL IMMUNE SERA

Mamta Sharma and S Rathaur
Department of Biochemistry, Faculty of Science, B.H.U., Varanasi.

The presence of SOD, an antioxidant enzyme in filarial parasite is well established. Significant amount of activity was estimated in adults and microfilarial stage of bovine filarial parasite *Setaria cervi*. The enzyme secreted during *in vitro* maintenance of parasite suggests that SOD may play an important role in parasitic defence. In order to pursue the role of SOD, enzyme was partially purified from adult male *S. cervi* by DEAE-sepharose CL-6B followed by sephadex G-100 column chromtography. Partially purified enzyme was immunologically reactive to *Wuchareria bancrofti* asymptomatic microfilaraemic carriers, elephantiasis and antibodies to recombinant *Brugia malayi* SOD. The purified IgG, IgM from *W. bancrofti* infected sera and rec. *B. malayi* SOD antibodies were used for *in vitro* inhibition of *S.cervi* SOD. When 20 µg protein of both partially purified SOD and different types of antibodies were incubated for 2 hr. at 37°C, the percentage inhibition of enzymatic activity was 68 with IgG & 34 with IgM in asymptomatic microfilaremic sera, where as in elephantiasis sera there was 56 & 14 per cent inhibition with IgG & IgM classes of antibodies respectively. Recombinant *B.malayi* SOD antibodies have shown 65% inhibition when 10 µg of both the preparations were incubated for 2hr. at 37°C. Filarial SOD specific IgG antibodies inhibited *S. cervi* SOD activity significantly. Thus *S.cervi* SOD could be a target for vaccine studies against filariasis.

O - 10.9

APPLICATION OF SEROEPIDEMIOLOGY IN IDENTIFICATION OF MALARIA ENDEMICITY IN SHANKARGARH, INDIA.

Arati Roy[1] and P K Tyagi[2]
1. Malaria Research Centre, Delhi.
2. MRC, Rourkela - 769 004

The value of seroepidemilogy in malaria control programmes has long been recognised, but its use in such situations have been limited. We therefore, conducted a sero epidemiology study in Shankergarh of Allahabad district. Shankargarh, malaria is highly endemic. The first serology survey was done in 1989 (transmission season), second was done in 1990 (non-transmission season) and the third was done in 1992 during non transmission season. The filter paper blood was examined using an ELISA test against a synthetic nonapeptide (AR-1). There was no difference in antibody titre between experimental and control village of Shankargarh during the dry season. There was a significant difference in antibody responses in malaria clinic patients in the year 1989 and 1992. The difference in the geographic distribution of high and low titres was indicative of permanent foci of malaria transmission in riverine villages of Allahabad district. Results of parallel parasitological study were consistent with those of the serologic study. A comparison of antibody titres against (AR-1) in all the age groups under varying malariogenic conditions showed difference in antibody response according to the status of malaria endemicity. These findings may be of practical value in the proper reorientation of the local malaria control programme.

O - 10.10

ADHERENCE OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES TO ICAM-1 : A MARKER OF PARASITE VIRULENCE

S M Handunnetti[1], P P S L Pathirana[1], R J Howard[2] and K N Mendis[1]
1. Malaria Research Unit, University of Colombo, Colombo, Sri Lanka.
2. Department of Molecular Biology, DNAX Research Institute, Palo Alto, CA, USA.

The expression of erythrocyte surface antigens of *Plasmodium falciparum* trophozoite-infected erythrocytes (IE) of parasite isolates from 44 uncomplicated (UC) patients from Sri Lanka was studied; cytoadherence properties of these 44 isolates were compared with parasite isolates from 12 severe and complicated (SC) patients. Erythrocyte surface antigens were expressed on *P. falciparum*-infected erythrocytes in almost all (100 & 96%) Sri Lankan parasite isolates as detected by either antibody mediated agglutination or surface immunofluorescence assay respectively. The intrinsic adherence and rosette forming properties of these isolates showed a wide phenotypic variation. Adherence of IE of all isolates to CHO-ICAM-1 and CHO-CD36 cells was significantly correlated ($r=0.3$; $p=0.04$) although neither properties correlated with the degree of rosetting.

In the UC malaria patients, the intensity of clinical disease (as measured by a clinical score) was significantly and positively correlated with IE adherence to ICAM-1 (r=0.54; p=0.003), but not to IE adherence to CD36 or degree of rosetting; this relationship was independent of parasitaemia in the patient (p=0.016, multiple regression analysis). Both IE adherence to CHO-ICAM-1 cell and the clinical scores of uncomplicated infections were significantly correlated with the percentage parasitaemia in the peripheral blood of these patients (r=0.36; p=0.025 and r=0.39; p=0.037 respectively). Secondly, IE adherence to ICAM-1 was found to be significantly higher in the SC isolates than in the UC isolates (p=0.007; z=2.67) whereas IE adherence to CD36 and rosetting were comparable among the two groups. These results indicate that IE adherence to ICAM-1 is associated with severity of clinical disease in *P. falciparum* malaria, whether it has a causative role or is a marker to parasite virulence remains to be further investigated.

O - 10.11

ANTIGENIC POLYPEPTIDES OF F1 FRACTION OF PROTOSCOLECES OF *ECHINOCOCCUS GRANULOSUS*

Gul Ahmad and W A Nizami
Section of Parasitology, Department of Zoology, A.M.U., Aligarh-202 002 (U.P.), India.

The partially purified F1 fraction of the protoscoleces fromt he column chromatography (Sephadex G-200) was subjected to immunoaffinity chromatography on a Sepharose 6B matrix. The polypeptide profile of the F1 fraction were further analysed by SDS-gradient polyacrylamide gel electrophoresis (PAGE). A total of 26 different polypeptides were resolved by Coommassie Brilliant Blue staining. Most of the polypeptides were of high molecular weight. The apparent molecular weight of these polypeptides are 125, 115-118, 90, 70, 68, 64, 57, 51, 49, 38, 32 and 29 kDa respectively. Some low molecular weight (<29 kDa) polypeptide were also resolved. The antigenicity of these polypeptides was investigated by immunoblotting using 24 and 40 days post infection dog antiechinococcus antisera. The results of the immunoblot analysis revealed that 7 and 6 polypeptides were found antigenic. The 24 day Pi sera detected polypeptides of Mr. 150, 118, 102, 74, 52.5, 48 and 35 kDa and 40 day pisera recognized the polypeptides having Mr. of 140 , 128, 102-105, 96, 71 and 51 kDa respectively. Among these antigenic polypeptides 102-105 kDa is commonly detected by both sera having some immunodiagnostic potential during the prepatent period (24-40 day post infection) before the worms attain maturity.

O - 10.12

AMA-1 OF P. FALCIPARUM AND P. VIVAX : RECOMBINANT EXPRESSION FOR SUB-UNIT VACCINE DEVELOPMENT AND HETEROLOGOUS EXPRESSION THROUGH TRANSFECTION OF MALARIA PARASITES

Alan Thomas[1], Annemarie van der Well[1], Jack Pronk[2], Martin Dubbeld[1], Andrew Waters[3], Chris Janse[3] Jan langermans[1] and Clemens Kocken[1]
1.Dept. Parasitology, Biomedical Primate Research Centre, Lange Kleiweg 157, 2288GJ Rijswijk, The Netherlands.
2. Kluyver laboratory of Biotechnology, Industrial Biotechnology Section, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands
3. Department of Parasitology, University of Leiden, Wassenaarseweg 62, 2300 RC Leiden, The Netherlands.

The Apical Membrane Antigen-1 (AMA-1) family of moleculesare merozoite antigens of undetermined function that apear to be intimately involved in red cell invasion. Two foms of AMA-1 have been described, an 83kDa form present in *P. falciparum* and the closely related chimpanzee mala *P. reichenowi* and a 66 kDa form that is present in all other malaria species analysed to date. The difference in mlecular weight is accounted for primarily by an insertion/deletion in the N-terminal region of the molecule. In simian and mouse models AMA-1 vaccination has induced protective immune responses, although there is evidence from the mouse model that regionally localised point mutations may have an impact on vaccine outcome. From vaccine studies and *in vitro* assays of parasite on multiplication in the presence of antibodies to AMA-1, it appears that the conformation of AMA-1 is critical to protective effects. Di-sulphide bonds have recently been assigned and a protocol to refold prokaryotically expressed *P. falciparum* AMA-1 is currently being used to generate material for clinical trials. Another approach is to use eukaryotic expression systems which do not require refolding. We have used the yeast *Pichia pastoris* for level expression of secretion products of the *P. vivax* 66kDa AMA-1 ectodomain (PV66/AMA-143-487) which do not require refolding. Purified PV66Dglyc43-487 is highly immunogenic in rhesus monkeys in combination with the adjuvant SBAS2.

To develop systems to better analyse, for example structural and functional relationships we have used AMA-1 as a leading molecule. We have transfected the rodent malaria *P. berghei* with the AMA-1 of *P. falciparum* (PF83/AMA-1). Stable expression of correctly folded PF83/AMA-1 has been obtained with a stage specific and subcellular distribution that strongly depends on promoter control. Using the AMA-1 promoter of *P. berghei.* to drive PF83/AMA-1 expression resulted in a sub-cellular distribution of the molecule similar to that seen in a *P. falciparum,* indicating that sequences influencing localisation are conserved between species. Processing of PF83/AMA-1 also occurs in *P. berghei.*. Mice infected with the transfected *P. berghei* produce high levels of PF83/AMA-1 specific antibodies. Similar results have been obtained by trnasfection of the macaque monkey malaria parasite *P.* knowlesi with heterologous AMA-1 species.

This shows that heterologous expression of critical malaria molecules between phylogenetically distant parasites is feasible and holds promise for a deeper understanding of structural and functional relationships for critical vaccine and drug targets.

O - 10.13

ARRESTED DEVELOPMENT OF *HYMENOLEPIS DIMINUTA* IN RATS FOLLOWING TREATMENT WITH SALICYLIC ACID

Souvik Ghosh, Arpita Saha, Santi P Sinha Babu, Nirmal C Sukul
Department of Zoology, Visva-Bharati University, Santiniketan - 731 235, West Bengal, India

Salicylic acid (SA) was first isolated from extracts fo different plants including willow. This drug has been used for relieving pain in man. The mode of action of SA is not yet fully understood. The latest finding that it plays a role in disease resistance response in plants, raises the possibility of some parallel action in animals too. This has prompted us to investigate the effect of SA on *H.diminuta* in rats. Salicylic acid was administered orally to a batch of 10 albino rats at the rate of 100 mg/kg body wt. for 7 days prior to their inoculation with cysticercoids. The rats were infected with a single cysticercoid of *H. diminuta* after treatment. The eggs did not appear in the faeces of the rats treated with salicylic acid. There was no appreciable change in the mean egg counts in the untreated rats. No adult worms were recovered from the treated rats on autopsy. Adult worms recovered from the control rats were found to be actively motile. The roel of salicylic acid on the development of *H. diminuta* in rats has been discussed.

O - 10.14

EFFECT OF PERMISSIBLE ADJUVANTS (NOR MDP, LTP) IN LIPOSOMES ON MALARIA VACCINE DESIGN

D N Rao[1], Bhavna Chaba[1], Pawan Kumar[1] and W Haq[2]
1. Department of Biochemistry, AIIMS, New Delhi-110 029.
2. Department of Biopolymers, CDRI, Lucknow.

Global resurgence of *P. falciparum* malaria, the most lethal of human malaria has led to intensified efforts to develop a vaccine against this disease. Recent advances in immunology, molecular biology and peptide chemistry have allowed the construction of subunit vaccines. The present study has been undertaken to overcome the major disadvantage of subunit vaccines i.e. their low immunogenicity and genetic restriction at the host level. Two permissible adjuvants nor-muramyl dipeptide and lauryl tetrapeptide (nor MDP & LTP) along with liposomes have been used to overcome the above limitations to generate a secondary response and long lasting immunity. Two synthetic peptide sequences (EENVEHDA)2 and (DDEHVEEPTVA) 2 from an asexual blood stage antigen, Ring infected Erythrocyte Surface Antigen (RESA) were linked to a promiscuous T cell epitope CST3 and were entrapped in liposomes (FTMLVs) along with nor

MDP/LTP. These antigen preparations adsorbed on alum were administered in four inbred strains of mice of different genetic background to study the humoral response. The results obtained showed that immunization with antigenic peptides along with nor MDP/LTP entrapped in liposomes generated high titre antibodies in comparison to the peptides in the absence of these adjuvants and the antibody levels persisted for longer duration. Further, the entrapment of antigen in liposomes reduced the immunizing dose fivefold as compared to conventional alum based preparations. The IgG isotype profile indicates a rise in IgG2a, 2b antibodies only suggestive of $Cd+_4$ TH_1 type response. These antibodies are shown to be protective during *in vitro* assay. This study highlights the importance of permissible adjuvants in conjunction with liposome delivery as an approach for peptide based vaccines against malaria.

O - 10.15

CHARACTERIZATION OF THE COMPONENT RESPONSIBLE FOR ANTIBODY MEDIATED DRUG TARGETING IN MALARIA INFECTED ERYTHROCYTES

Grish C Varshney and Amit Choudhury
Institute of Microbial Technology, Chandigarh - 160 036, India

The existence of neoantigenic determinants on the surface of malaria infected erythrocytes in different species has been adequately validated. These new antigens can be potential targets for : i) the development of vaccines, ii) site specific drug targeting and iii) delineating structure-function relationships in malaria infected erythrocyte membranes. Rodent model provides a good system for elucidating the functional significance of such antigens. In our recent study we demonstrated that chloroquine (Chq) encapsulated in malaria-infected erythrocyte-specific antibody ($MAbF_{10}$) bearing liposomes effectively controls Chq resistant *Plasmodium berghei* infection in the mice. In this study, we have further studied the kinetic pattern binding and uptake of ^{125}I-$MAbF_{10}$ in infected erythrocytes and parasites under conditions that permit (37°C) or block (4°C) endocytosis. The antigenic determinant responsible for functional drug targeting was also characterized. As revealed by immunofluorescence assay, immunoelectron microscopy and flow cytometry and binding sites of $MAbF_{10}$ were localized on infected cell surface, host cytoplasm and on intracellular parasite surface. The > 205 kDa antigen, recognized by the antibody was present on intact cell surface, as revealed by immunoprecipitation. [^{35}S]-methinonine labeling indicated the parasitic origin of the protein. Studies are underway to further characterize this protein.

O - 10.16

CYTOKINE PRODUCTION BY INTERVILLOUS BLOOD MONONUCLEAR CELLS: THE INFLUENCES OF GRAVIDITY AND PLACENTAL MALARIA

Julie M Moore[1] , Bernard Nahlen[1,2] ,Josephine Njeri[2], Ben Wachiri[2], Altaf Lal and Venkatachalam Udhayakumar[1]
Division of Parasitic Diseases, NCID,CDC, Atlanta, GA,USA.
VBCRC,Kenya Medical Research Institute, Kisumu, Kenya.

Placental malaria, especially in high transmission areas, occurs more frequently in primigravid and secundigravid than in multigravid women. Although this finding has been confirmed in several epidemiological studies , it is not known how multigravid women are protected against placental malaria. Some authors have proposed that exposure to malaria through successive pregnancies can induce placenta-specific immunity capable of controlling placental parasitemia. Howere, no major attempt has yet been made to determine the nature of immunity at the placental level. We recently developed a perfusion method which allows us to isolate maternal intervillous blood mononuclear cells (IVBMC) from term placentae.Because cytokines are thought to play an important role in anti- malarial immunity,we examined the cytokine production profiles of IVBMC isolated from placentae of women residing in a holoendemic area of western Kenya .Supernatants from 48 hour cultures were assayed for IL-2,IL-4,IL-10,IFN-$_r$ and TNF-$_\alpha$ production in response to stimulation with phytohemagglutinin (PHA), anti-CD3 monoclonal antibodies (CD3)and *P.falciparum* exoantigens. The most notable responses were seen for IL-4,IFN$_r$and TNF-$_\alpha$ .Most sample producedIFN-$_r$andTFN-$_\alpha$ enogenously, whereas spontaneous IL-4 production was much less frequently observed. Likewise, in response to PHA andCD3 stimulation, most samples responded with vigorous IFN-$_r$ production ,while relatively modest and infrequent IL-4 responses were seen. Thus , these data suggest that IVBMC may be biased toward Th1- type cytokine responses,which is in contrast to the Th2- type cytokine bias reported for murine placenta. For all stimulants, there was a trend toward increased IFN-$_r$ production in multigravid versus primigravid IVBMC.In contrast , IL-4 production in response to exoantigens was more frequent and higher in primigravidae than in multigravidae. Finally, production of both IL-4 and IFN-$_r$ declined in the presence of placental parasitemia .In summary, it appears that IVBMC tend toward production of Th1-type cytokine production ,particularly IFN-$_r$and this bias becomes stronger with increasing gravidity. In addition ,changes in cytokine production profiles are evident in association with placental malaria.These gravidity- and parasitemia-associated differences in the placental cytokine environment may provide an explanation for the reported differential susceptibility to placental malaria infection among women of different gravidities.

O - 10.17

IGE IN *P. FALCIPARUM* MALARIA, A TH2-TYPE OF IMMUNE RESPONSE AND IT'S POSSIBLE PATHOGENIC ROLE

Gehad ElGhazali§*, Hedvig Perlmann*, ASM Rutta, Peter Perlmann* & Marita Troye Blomberg*
§ Faculty of Medicine, University of Khartoum, Sudan, *Department of Immunology, Stockholm University, 106 91 Stockholm, Sweden. National Institute for Medical Research, Amani, tanzania.

People living in *Plasmodium falciparum* endemic areas frequently have elevated levels of total as well as *P. falciparum* specific serum IgE. Elevated IgE levels suggest an immunoglobulin switch from IgM caused by the "switch factors" IL-4 and IL-13 released by activated Th2-cells. To investigate the role of Th1 and Th2 cells in the human *P. falciparum* system we used the ELISPOT assay to determine the ratio of IFN-γ and IL-4 producing cells after specific antigen or mitogen activation *in vitro*.The donors were individuals who had acquired immunity through natural exposure to the malaria parasite *P. falciparum*. The anti-malaria IgE levels in serum correlated with an increased ratio of IL-4/IFN-γ producing cells. Thus, donors with ratios of IL-4/IFN-γ > 1 exhibit mean serum anti-malaria IgE levels significantly greater than those with ratios < 1. Our data suggest a skewing in the balance of $CD4^+$ T-cells towards the T helper 2 subset in naturally *P. flaciparum* primed individuals, associated with elevated serum levels of anti *P. falciparum* IgE levels. Notwithstanding its potential protective capacity. IgE very likely also contributes to the pathogenesis of *P. flaciparum*. This is supported by the finding that IgE elevated is consistently highest in cerebral malaria. IgE containing immune complexes may give rise to local over-production of TNF by monocytes through cross-linking of CD23, the low-affinity receptor for IgE. This is supported by 1) the *in vitro* induction of TNF in adherent blood mononuclear cells from normal donors after exposure to IgE containing malaria sera in the presence of anti-IgE antibodies and 2) the positive correlation between IgE and TNF levels in patients with cerebral malaria. Thus, IgE and the underlying skewing of the Th2/Th1 balance are contributing to the pathogensis of malaria.

O - 10.18

ANTIBODIES TO A NON-REPEAT SEQUENCES OF *PLASMODIUM FALCIPARUM* ANTIGEN Pf155/RESA PREVENT CYTOADHERENCE AND MEROZOITE INVASION *IN VITRO*

A B Siddique, N. Ahlborg, B. Wahlin Flyg, P. Perlmann & K. Berzins.
Department of Immunology, Stockholm University, S-106 91 Stockholm, Sweden

In *Plasmodium falciparum* malaria, antigens derived from surface of infected erythrocytes or parasites play important roles in either the cytoadherence or merozoite invasion process. It has previously been shown the non-repeat region of antigen Pf155/RESA

contains a hexapeptide motif being highly homologous to a cytoadherence related sequence of erythrocyte band 3. We now report antibodies to multiple antigenic peptides (MAPs) containing this homologous hexapeptide motif from either Pf155/RESA or band 3 inhibit the binding of *P. falciparum* infected erythrocytes (Palo Alto strain) to melanoma cells *in vitro*. Furthermore, rabbits immunized with these MAPs generated antibodies showing specific reactivity with band 3 and Pf155/RESA respectively, in immunoblotting and immunofluresence, and the antibodies also inhibited cytoadherence of *P. falciparum*infected erythrocytes of melanoma cells *in vitro*. While antibodies to the hexapeptide motif did not interfere with parasite growth *in vitro*, antibodies to neighbouring sequences (residues 199-214) inhibited merozoite invasion of both $RESA^+$ amd $RESA^-$ parasite *in vitro*. Our results indicate that antibodies to epitopes formed by these non-repeat sequences of Pf155/RESA may mediate parasite neutralization and cytoadherene inhibition, and thus, the immunogens based on these sequences are of interest for development of *P. falciparum* malaria vaccines.

O - 11.1

THE GEOGRAPHICAL DISTRIBUTION AND DRAMATIC INCREASES IN INCIDENCES OF MALARIA : CONSEQUENCES OF THE RESETTLEMENT SCHEME IN GAMBELA, SW ETHIOPIA

Mengistu Woube
Geographics Laboratory, Uppsala University, Sweden 752 36 Uppsala.

The spatial distribution of malaria results from the interaction between vector, parasite, host, physical and human environments. This basic geographical approach provides an illustration of the geographical distribution of malaria in the world, particularly in the tropical regions. Although national governments, international organisations and scientists are involved in trying to control malaria, millions of people suffer from this disease in developing countries, particularly in the tropical regions of the world. Due to the global climatic change and population movements, it is predicted that malaria could have a great impact on the non-immune or unprepared populations in the Northern Hemisphere in the coming decades. Presently, Sub-Saharan Africa (SSA) is the most affected region in the world. Like any other SSA country, the Ethiopian people suffer from both epidemic (unstable) and endemic (stable) malaria in the highlands and the lowland regions, respectively. Gambela is one of the areas with stable malaria in the humid tropical region of the country.

This study is based on observations, unpublished data, interviews and discussions with settlers and officials in Gambela. It is found out that a degree of diverse malaria prevalence is associated with altitudinal, temperature and rainfall variations. Owing to the settlement and land-use changes, unexpected rainfall patterns, temperature increase, unstable political system and poverty, malaria has gone beyond its geographical limits. As a result, the number of malaria affected people has increased in the last 12 years. When Gambela was settled by a small number of indigenous people and when there was hardly any development programme, malaria was not a major problem. Following the 1970s land reform programme, the 1980s resettlement scheme and the introduction of large-scale farms and rapid unplanned urban growth, malaria has affected thousands of people. It has also brought serious obstacles to the implementation of other developmental programmes and on the region's health. In short, though the natural resources pulled (attracted) government planners and people (resettlers), malaria has now become one of the main pushing factors. It is suggested that proper physical and social planning, understanding the geography, entomology, epidemiology, behaviour and life-cycle of malaria, co-operation between the policy-makers, malaria specialists, neighbouring countries and international communities are urgent, if malaria can be controlled and eradicated.

O - 11.2

APPLICATION OF GEOGRAPHIC INFORMATION SYSTEM TO DELINEATE POTENTIAL AREAS FOR MALARIA VECTOR OCCURRENCE

Aruna Srivastava, B N Nagpal, Rekha Saxena and V P Sharma
Malaria Research Centre, 20 Madhuban, Delhi 110092

In India , out of 58 anopheline mosquito species only 10 have been reported as the primary and secondary malria vectors. The breeding and longevity of vectors are the functions of ecological conditions which in turn establish there population in an area. Thematic layers of India in the scale of 1: 600 000 of soil, physiographic, monsoon/ rainfall, teerature, forest and urban areas were used to delineateotential areas of six major vector distribution. The analysis was done using software ARC/ INFO 3.1. Each themmap was stratified in to favorable, moderately favorable and unfavorable zones in respect of breeding and sural. Known features of vector biology and naturally occurng distribution constituted base line information. Overlaythematic maps and analysis of contours formed by union and intersection of favorable and unfavorable zones in individuals thematic layers provided information on probability of occurrence of each vector species. Study was helpful in delineating possibel vector distribution in unsurveyed areas for planning vector control to interrupt malaria transmission.

O - 11.3

AN ANALYSIS OF THE ANIONS IN THE MICROENVIRONMENT OF THE CERCARIA OF *FASCIOLEPSIS BUSKI* AND THEIR HOST SNAIL.

Soam Prakash and Anu Kumar
Environmental Parasitology Laboratory, Department of Zoology, Faculty of Science, Dayal Bagh Educational Institute, Dayalbagh, Agra, 282 005

The habitat and environment of Cercaria's of *Fasciolepsis buski* and that of the snail host itself have been under intensive investigation for various purposes including vector control, diagnosis, communication, host localization, ecology and micro manipulations. During the present study, the role of anions, including chloride, sulphate, phosphate, fluoride, etc. of cercarias, and their various snail hosts have been investigated to validate the presence of an anion channel by the Dionexion chromatography to ascertain the role of anions in the formation of microenvironment. Their specific concentration, depicts and signify requirement that can result into further utilization, will be discussed.

O - 11.4

OBSERVATIONS ON SOME OF THE VITAL PHYSICO-CHEMICAL FACTORS ON THE EMERGENCE OF CERCARIAE

J P Yadav and A K Maurya
Parasitology and Ecology Laboratory, Department of Zoology (CAS), Banaras Hindu University, Varanasi- 221 005, India.

Effect of some of the vital physico-chemical factors like temperature, pH, dissolved oxygen, free carbon dioxide and calcium were evaluated on the emergence of cercariae from fresh water snail hosts of pond at Jaunpur (India) during the period June, 1995 to May, 1996. During this period considerable changes in the quality of pond water were observed. Two peaks of cercarial emergence was noticed from both the snails *Indoplanorbis exustus* (Deshayes)
and *Lymnaea luteola F.* typica Lamark. In *Indoplanorbis exustus,* first peak of 83.9% was observed in the month of October and second peak of 25.0% in the month of April. Similarly in *Lymnaea luteola* first peak of 35.71% in the month of September and the second peak of 16.7% in the month of May and no cercarial emergence was noticed in the month of June. However, no cercarial emergence was noticed in the snail *Vivipara bengalensis* throughout the study period. In the present finding it has been observed that temperature and pH play a potent role in the cercarial emergence from snail hosts but dissolved oxygen, free carbon dioxide and calcium were not found to have any significant role.

O - 11.5

INCIDENCE OF ENDO AND ECTO PARASITES OF SHEEP (*OVIS ARIES*) IN ANDHRA PRADESH (INDIA)

N Srinivasa Rao and Md Hafeez
Department of Parasitology, College of Veterinary Science, Acharya N. G. Ranga Agricultural University, Tirupati - 517 502 (A.P.) India

The incidence of parasites and parasitic infections / infestations was studied in nine selected places representing three different agroclimatic regions in Andhra Pradesh. Among a total of 2674 of sheep examined, 84.87 ± 2.03 percent of animals had one or other parasitic infection / infestation. The percentage of incidence of helminth, protozoan and arthropod parasites was 76.69 ± 4.81 and 74.38 ±3.66 per cent, respectively.

The study revealed the incidence of 43 species of parasites comprising of trematodes viz. *Fasciola gigantica,_Paramphistomum epiclitum, Gastrothylax crumenifer, Fischoederius elongatus, Fischoederius cobboldi, Ceylonocotyle thapari, Calicophoron calicophorum, Calicophoron, cauliorchis,* Cestodes viz, *Moniezia expansa, Moniezia benedeni, Hydatid cysts, Cysticercus tenuicollis, Cysticercus ovis, Coenurus cerebralis, Avitellina Centripunctata, and Stilesia hepatica,* Nematodes *viz., Haemonchus contortus,*

Trichostrongylus colubriformis, Oesophagostomum columbianum, trichuris ovix, Bunostomum trigonocephalm, Chabertia ovina, Mecistocirrus digitatus, and Strongyloides papillosus, Protozoan parasites *viz., Eimeria arloingi, Eimeria faurei, Eimeria parva* and other *Eimeria* sps. The haemoprotozoan parasite recorded was *Theileria* and the tissue protozoa encountered was *Sarcocystis* sps. The arthropod parasites that were observed included the lice *Linognathus vituli* and *Damalina ovis,* ticks belonging to the different species *viz. Haemaphysalis intermedia, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Boophilus microplus* were recorded. Psoroptes mites, were also encountered and occasionally the flea *Ctenocephalides felis* was found. The flies *Culicoides* and *Musca* were also recorded on some sheep.

O - 11.6

M Rokkam
(Abstract not received)

O - 11.7

A COMPARATIVE STUDY ON THE GROWTH AND DEVELOPMENT OF *SCHISTOSOMA INCOGNITUM* / (CHANDLER, 1926) / AND *SCHISTOSOMA SPINDALE* / (MONTGOMERY, 1906).

U Shameem
Dept. of Zoology, Andhra University, Visakhapatnam, A.P., India.

Growth patterns and developmental stages were traced for two sps. of *Schistosoma* employing mouse an experimental host and cercariae collected from naturally infected snails *Lymnaea luteola* and *Indoplanorbis exustus*. A set of 20 BALB/C strain mice were exposed to each sps. of cercaria and were sacrificed at regular intervals to follow different growth and developmental stages. Flukes recovered each stage were processed and details were studied. Both the sps. exhibited three phases of growth *viz.* a lag-phase during which there is no growth, a rapid growth phase characterised by an increase in growth, rate and a stationary phase during which growth is either limited or totally absent. Studies on different developmental stages, revealed five stages viz., a non-differentiation stage, gonadial differentiation stage, pre-ovigerous stage, ovigerous stage and an embryonated egg producing stage. The time duration of each stage varied with respect to each sps. In case of *S. incognitum* the flukes reached maturity at 40 DPI and eggs can be detected in the faeces. Whereas *S.spindale* carried only immature eggs in the uterus even at 85 DPI. Differences were also noted in the frequency of pairing of male and female flukes, body growth and organogeny.

O - 11.8

SEASONAL INCIDENCE OF CLINOSTOME METACERCARIAE IN *CHANNA GACHUA* AT MANSAR JAMMU

Ranvijay Singh Salaria, M K Jyoti and P L Duda
Department of Biosciences, University of Jammu, Jammu-180004, J&K, India.

The incidence of infection of the metacercariae of *Clinostomum giganticum* and *Euclinostomum reticulatum* in *Channa gachua* has been studied for two years in succession at a freshwater lake Mansar in Jammu. Significant seasonal variations were evident; *Clinostomum giganticum* me tacercariae were significantly more prevalent during the months of May, June and July when the atmospheric temperature is on the rise, whereas that of *Euclinostomum reticulatum* metacercariae was significantly higher during the months of October, November and December when the ambient temperature is on the decline indicating thereby a district seasonal segregation of the two infecting taxa in the host. Moreover, the two metacercariae were never found to coexist in the same host specimen despite their simultaneous occurrence in the water body. The adaptive significance of this segregation has been discussed in the paper.

O - 11.9

B Colin
(Abstract not received)

O - 12.1

COMPARISON OF FIELD'S STAIN AND FLUORESCENT DYE (ACRIDINE ORANGE) FOR THE EFFICIENT DETECTION OF PLASMODIUM INFECTION

Parijat N Goswami, Jayshree Patel, Parul Pandit
Division of Microbiology, The Gujarat Cancer & Research Institute, Ahmedabad

Field's stain a quick staining method for Malarial parasites is the method adapted as a routine practice in the haematology laboratory of the Gujarat Cancer & Research Institute.

In case of scanty parasitemia, it becomes a tiring job and a tendency to miss the Malarial parasites for a microscopist during the procedure of screening. To overcome this, we planned to stain the Malarial parasites with a Fluoroscent dye Acridine orange. Acridine orange has marked affinity for nucleic acids. The RNA components flouresce oranish red and the DNA components yellow green. Eventhough, this method is used to study the virus growth in the host cells and for revealing scanty bacteria in wet films in blood culture we adapted this to detect Malarial parasites in the whole blood.

A study of 50 cases of whole blood from Blood Bank and Haematology Department were screened by both the staining methods i.e. fields stain and fluorescent dye, over a period of 3 months from May '97 to July '97. We also used a new, rapid & easy to perform test called Parasight F, for the evaluation of the samples collected. The results will be analysed statistically and presented during the conference.

O - 12.2

CYTOCHEMISTRY AND ULTRASTRUCTURE OF THE EXCRETORY SYSTEM OF THE METACERIA OF *CLINOSTOMUM COMPLANTUM*

P N Sharma and Purnima Meiwal
Department of Zoology, Mohan Lal Sukhadia University, Udaipur 313 001.

Cytochemistry and fine structure of the excretory duct, collecting duct and excretory bladder have been studied in metacercaria of *Clinostomum complantum*. The entire excretory system is lined with the syncytial epithelium which however, exhibit differences in thickness in different regions. The syncytial epithelium bears numerous surface lamellae of various sizes. The epithelium of excretory duct and that of collecting ducts are connected with parenchymal cells by gap junctions. The morphological characteristics of excretory ducts have been considered to be conducive for the transport of metabolites. The role of Acid phosphatase and Adenosine triphosphatase has been discussed in relation to the translocation of metabolic substances. The physiological significance of lipids and calcareous corpuscles in association with excretory role has been indicated. The ultrastructure of the calcareous corpuscles in the fixation of CO_2 and in

buffering of acids has been discussed. Occurrence of calcareous corpuscles appears to be the outcome of their immediate niche.

O - 12.3

ULTRASTRUCTURE OF THE DIGESTIVE AND PROTONEPHRIDIAL SYSTEMS OF THE METACERCARIA OF *EUCLINOSTOMUM MULTICAECUM* (EUCLINOSTOMATIDAE : DIGENEA) FROM THE LIVER OF THE FRESHWATER FISH *CHANNA STRIATUS*.

G P Brennan[1] and P Ramasamy[2]

1. School of Biology and Biochemistry, The Queen's University of Belfast, Belfast BT9 7BL U.K.

2. Department of Zoology, Life Science Building, University of Madras, Madras-600 025, India.

The digestive tract of *Euclinostomum multicaecum* consists of an oral sucker, a mouth and an acetabulum, but no recognised pharynx was observed. The surface tegument extends into the oral cavity, the oesophagus and the acetabulum. The gut comprises 8-12 main lateral caeca which extend posteriorly and further divide giving rise to numerous smaller branches which are widely distributed throughout the fluke. The gut caecal wall consists of a syncytial gastrodermal epithelium bearing loop-like lamellae which enclose spherical vesicles and, presumably, increase the absorptive surface area. These vesicles may represent storage sites for nutrients or they may have a role in the encystment of the metacercariae. Endo-and exocytosis were not evident nor were lysosomes, phagosomes and residual bodies. The gut caeca are enclosed by layer of muscle fibres supported on fibrous basal lamina while parenchymal cells, occupying much of the extracellular space, were distinguished by their substantial quantities of glycogen.

The protonephridial system consists of flame cells and collecting ducts which join to form a bladder that opens through a single excretory pore. The flame cell contains a core of 46/100 flagellae each consisting of 9 + 2 axial filament arrangement. The syncytial epithelium of the collecting ducts is elevated by numerous lamellae. Prominent nuclei and numerous mitochondria are also present. The luminal contents of these ducts comprise bar-shaped crystalline structures, lipid droplets and electron dense inclusion bodies. The excretory system is supported by a network of muscle fibres and parenchymal cells.

O - 12.4

ELECTRON IMMUNOCYTOCHEMICAL DEMONSTRATION OF NEUROPEPTIDES IN THE NERVOUS SYSTEM OF *PRICEA MULTAE* (POLYOPISTHOCOTYLEA : MONOGENEA).

P Ramasamy[1], G P Brennan[2], A G Maule[2] and D W Halton[2]
1. Department of Zoology, Life Sciences Building, University of Madras, Guindy campus, Madras-600 025, India
2. School of Biology and Biochemistry, Medical Biology Centre, Queen's University of Belfast, Belfast BT9 7BL U.K.

The nervous system of *Pricea multae* is well developed. It consists of both central nervous system and peripheral nervous components which extend the full length of the body. The haptor is richly supplied with nerve fibres which in turn are non-myelinated and contained numerous membrane bound small electron dense granules; small dense-cored vesicles and contained mitochondria. Similar dense cored vesicles were noticed in some of the neurons. In these vesicles, the subcellular localisation and distribution of NPF and FMRP amide-like immunoreactivity within the nervous system of *P. multae* has been determined by a post-embedding IgG conjugated colloidal gold immunostaining technique. Gold labelling of NPF/FMRP amide-like peptides were co-localised over dense-cored vesicles within nerve cell bodies and then processes constituting of the anterior ganglia, central commissures and nerve cords of CNS and PNS. Most of the gold label was concentrated over the contents of the dense core of the vesicles. No immunogold labelling was noticed in control section or in other types of vesicles or in any of the adjacent tissues.

O - 12.5

THREE-DIMENSIONAL ULTRASTRUCTURAL RECONSTRUCTION OF *PLASMODIUM FALCIPARUM* ASEXUAL STAGES

Lawrence H Bannister, John M Hopkins, Ruth E Fowler and Graham H Mithell
Departments of Anatomy and Immunology, UMDS, The Medical School, Guy's Hospital, London Bridge, London SE1 9RT, UK.

Our knowledge of *Plasmodium falciparum* ultrastructure is based mainly on random sectioning methods, but to understand the cellular organization of the parasite and its structural interactions with the host, three-dimensional reconstructions are needed. Using the approach, we have explored the structure of the asexual stages of this parasite, including rings, trophozoites, schizonts and merozoites. The method has provided much new information about the intracellular organelles within these stages including Golgi complexes, plastids and the parasitophorus vacuole-red cell relationship, and also throws light on the question of a possible parasitophorus duct.

O - 12.6

J Sattabongkot
(Abstarct not received)

O - 12.7

SURFACE FINE TOPOGRAPHY OF ADULT AND LARVAL STAGES OF *POLYSTOMA INDICUM* DIENGDOH ET TANDON, 1991 (MONOGENEA : POLYSTOMATIDAE)

M Dutta, V Tandon and R Imkongwapang
Department of Zoology, North-Eastern Hill University, Shillong - 793 022, India.

Scanning electron microscopic studies of the adult *Polystoma indicum,* the only hitherto known representative of the genus from amphibian hosts in the Indian subcontinent, has revealed that the tegumental surface is devoid of spines or scales but is coarsely covered with tegumental fold ridges and craters. Minute tubercles abounding all over the surface give it a beaded texture. Tuberculate elevations or the ridges possess pitted apices. Dome-shaped papillae, some pitted at their apices, encircle the oral sucker. The general tegument of the forebody and opisthaptoral disc has a fine tuberculated texture. The tegument of the larval stages also revealed the presence of folds and ridges giving the surface a honeycomb-like appearance. However, in the six-suckered post-gyrodactyloid-I stage tegumentary vesicles and button-like papillae are unevenly distributed and striae of anatomosing lamellae form concentric round patches with a cilium within each at several places. The surface of the prohaptor in all the stages shows domed papillae and that covering the opisthaptor and clamps appear spongy.

O - 13.1

COMPARISON OF INTRAMUSCULAR ALPHA-BETA ARTEETHER AND INTRAVENOUS QUININE IN THE TREATMENT OF CEREBRAL MALARIA

Saroj K Mishra, Sanjay K Satpathy, Sanjib Mohanty, Sanghamitra Satpathi and Jayakrishna Patnaik
Departments of Internal Medicine, Critical Care Unit & Pathology, Ispat General Hospital, Rourkela.

Alpha-beta arteether is an antimalarial derivative from artemisinine. Its efficacy in uncomplicated falciparum malaria has been documented in the form of rapid fever clearance and parasite clearance. In this study we compared its efficacy in the management of cerebral malaria. Two groups of such patients were compared; one group was treated with intramuscular arteether (150 mg once daily for 3 days, n = 26). Patients in the other group were administered intravenous quinine (n=22). There was no difference in the fever clearance time (Mean ± Sd being 74.2 ± 34.5 hrs for arteether and 83.78 ±50.47 hrs for quinine) or in the parasite clearance time. The prime observation in our study was the recovery of patients even from extremely critical situations. Recovery time from coma was rapid in patients receiving arteether (24.5 ± 14.7 *vs* 38.1 ± 26.8 hours, $p < 0.02$). Mortality was less in the arteether treated patients (3.85%) than the quinine treated patients (27.27%, $p < 0.02$). The side effects were significant with quinine therapy: two patients had hypoglycaemia and seven developed cinchonism during therapy.

O - 13.2

DIFFERENT TARGET GENE MUTATIONAL PATTERNS MEDIATING ANTIFOLATE RESISTANCE IN *P. FALCIPARUM.*

O Skold, and G Swedberg
Division of Microbiology, Department of Pharmaceutical Biosciences, Biomedical Center, Uppsala University, PO Box 581, S-751 23 Uppsala. Sweden.

Cycloguanil, pyrimethamine and sulfonamides interfering with folate metabolism in plasmodia have been used as antimalaria drugs for several decades. Cycloguanil and pyrimethamine are antifolates, competing with dihydrofolate in the dihydrofolate reductase reaction. Sulfonamide is a competitive inhibitor of dihydropteroate synthase (DHPS), which links dihydropteridine and para aminobenzoic acid to form dihydropteroate, which is the next to last metabolite in the sequence leading up to dihydrofolate. Mutations in the *dfr* gene were detected by a PCR method coupled with restriction enzyme digestion analysis. Nucleotide sequence determinations on the dihydrofolate reductase gene (*dfr*) of isolates of *P. falciparum* from different provinces of Vietnam collected on filter papers showed mutation patterns indicating a dominance of pyrimethamine resistance over cycloguanil resistance. This might lead to a reconsideration of the use of Paludrine. Mutations in the DHPS gene were detected by a PCR method using specific primers. Here the results were more varied and many samples

showed signs of simultaneous occurrence of both sensitive and resistant parasite. This was also borne out by nucleotide sequence determinations and by enzyme assays on plasmodium extracts. The presence of sulfonamide resistance mutations showed no clear correlations with the outcome of Fansidar treatment. In one Vietnamese province 38, patients were treated with Fansidar and at the same time blood samples were collected. Parasitemia was measured after 2 and 7 days. Only 11 of the treated patients showed clear signs of parasites after treatment, as measured by microscopy. This was surprising since all samples contained parasites with one mutation specific for sulfadoxine resistant isolates. All samples showed the presence of pyrimethamine resistance mutations.

O - 13.3

STUDIES ON PLASMODIAL HAME OXYGENASE, CALCIUM CHANNEL BLOCKERS AND ANTIDEPRESSANTS.

Pratima Srivastava, SK Puri*, KK Kamboj** and VC Pandey,
Division of Biochemistry, Microbiology* and Parasitology** Central Drug Research Institute, Lucknow - 226 001.

Verapamil, gallopamil, nicardipine, desipramine and chloropramazine (by virtue of their different properties as calcium channel blockers/antidepressants etc) have been clinically used to rectify many physiological disorders in human. In recent years they have also been used as resistant reversal agents against plasmodia. The mode of action of these compounds as resistant reversal agent may be due to their efficacy in preventing the efflux of chloroquine or interaction with plasmodial glycoprotein etc. Here we present a novel behaviour of above mentioned compounds in relation to parasite and corresponding normal and infected host HEME OXYGENASE. The results showed that verapamil and chloropromazine significantly inhibited heme oxygenase of the cell free rodent (*P. berghei / P. yoelii*) simian (*P. knowlesi*) and human (*P. falciparum/p. vivas*) malaria parasites. Kinetic studies of parasite's heme oxygenase had distinct dissimilarities from the host enzyme. It can be concluded that apart from the clinical efficacy, Ca^{2+} channel blockers, antidepressant etc. possess an additional unique property of inhibiting plasmodial heme oxygenase.

O - 13.4

COMPARATIVE TRIAL OF A NEW TREATMENT, CGP 56697 VS. CHLOROQUINE FOR TREATMENT OF ACUTE UNCOMPLICATED *P. FALCIPARUM* MALARIA IN ADULTS IN INDIA

N Kshirsagar, N Gopinathan, N Murthy, M Garg, S Dalvi, A Chogle, J Sorabjee, S Marathe, S Sane, A Bhatt, B. Gemperli, I Gathmann and C Royce.
KEM Hospital, Kasturba Hospital, Novartis Hinudstan, Novartis Basel Switzerland.

CGP 56697 is a combination of artemether with a novel compound, benflumetol. One

hundred and seventy-nine hospitalised patients (2 centres) with acute uncomplicated *falciparum* malaria aged > 16 years were randomised to receive either CGP 56697 (89 patients took 4x4 tablets, each tablet containing 20 mg artemether + 120mg benflumetol) or chloroquine (90 patients took 1x4 then 3x2 tablets, each talet containing 150mg chloroquine sulphate). Both drugs were taken over a 48 hours period. In-patient observation was for 8 days, total follow-up was 29 days. ECG's were performed on days 4,8 and 29.

CGP 56697 cured 62 of 65 evaluable patients (95.4% 28 days cure rate) whereas chloroquine only cured 15 of 76 evaluable patients (19.7% cure rate, $p < 0.001$). There were 3 failures on CGP 56697 : (all RI) as compared to 61 on chloroquine (49 RI, 8 RII and 4 RIII). Parasite reduction at 24 hours was 98.8% (n=89) with CGP 56697; 70.7% (n=89) with chloroquine. Time to fever clearance was 18 hours (n=40) with CGP 56697 and 27 hours (n=54) with chloroquine. Time to gametocyte clearance was 120 hours (n=51) with CGP 56697 and 336 hours (n=63) with chloroquine. All data refer to medians, n = number of evaluable patients.

QTc were not affected by either treatment. One patient on chloroquine died of cardio-respiratory arrest due to progression to cerebral malaria on Day 6. CGP 56697 was safe and well tolerated and associated with fewer adverse event (AE) reports (i.e. vomiting 1.1% vs 15.6%) than chloroquine.

This trial demonstrated that CGP 56697 provides a rapid, effective and safe treatment of acute uncomplicated *P.falciparum* malaria in adults in India. CGP 56697 is statistically significantly superior in efficacy compared to chloroquine (28 day cure rate 95% vs 19.7%).

O - 13.5

A NOVEL APPROACH TO THE TREATMENT OF ACUTE BLOOD-STAGE MALARIA : COMBINATION OF LOW DOSE CHLOROQUINE AND IL-12

Karkada Mohan and Mary M Stevenson
Montreal General Hospital Research Institute, McGill University, Montreal, PQ, Canada.

Development of resistance by *P. falciparum* to many antimalarials coupled with resurgence of disease in many developing countries makes it essential to develop new drugs or to modify current therapeutic strategies against this potentially fatal infection. Encouraged by our previous observation that 0.1 µg IL-12 treatment for 6 days results in up to 100% survival of *P. chabaudi* AS susceptible A/J mice, we examined the protective efficacy of combining low doses of chloroquine (CQ) and IL-12 to treat blood-stage infection. While untreated mice developed parasitemias > 50% and succumbed to infection, mice receiving half the therapeutic dose of CQ (total 12.5 mg/kg) had a peak parasitaemia of about 20% and survived infection. Combining this dose of CQ with half the protective dose of IL-12 (total 0.3 µg/mouse) resulted in a significant reduction in

parasitaemia to 3%, a longer prepatent period, a delayed peak parasitaemia and a survival rate of 100%. Preliminary findings also suggest that by reducing the dose of IL-12 further to 0.2 µg/mouse and starting treatment after the prepatent period, on day 3, the protective efficacy of this IL-12+CQ combination essentially remains the same as observed above. Moreover, mice receiving this therapy showed significantly higher hematocrits, red cell counts and percentage reticulocytes compared to untreated mice. This combined therapy may have several advantages : i) low dose CQ provides the opportunity to increase dosage if required, thereby prolonging the development of drug resistance; ii) a low protective dose of IL-12 may allow clinical use at non-toxic levels; iii) since IL-12 treatment can be started along with CQ after detection of patent parasitaemia, it may have applicability to clinical use; iv) since IL-12 can boost erythropoiesis during malaria, this therapy may prevent life threatening anemia associated with severe *P. falciparum* malaria; and v) as IL-12 induces a protective Th1 type host response against malaria parasites, following this combined therapy, the level of immunity developed might be relatively long lasting and protective against reinfection.

O - 13.6

PHASE III CLINICAL TRIAL OF ARTEETHER IN MALIGNANT TERTIAN MALARIA

Aspi Balsara
Department of Medicine, Tata Main Hospital, Jamshedpur, INDIA.

Total 158 cases of M T Malaria were included in the study. Out of which 58 cases were of uncomplicated and 100 cases of complicated M T Malaria. All the cases were administered 150 mg of Arteether IM/day for 3 days, after fulfilling the criteria of the protocol. Clinical Haematological and Biochemical Evaluation was done on the specific days. It was found that in uncomplicated cases the Arteether was very useful and effective drug controlling for fever and symptoms within 48 hours. It has no side effects and probably it also has the gametocytic effect in addition. In complicated cases the symptoms were relieved within 72 hours. The main complications noticed were Severe Anaemia, Cerebral Malaria, Renal Failure, Gangrene, Pneumonia and Haemolytic Jaundice. The overall mortality in the complicated M T malaria cases was 28%. The remaining 72% recovered. Some of them required supportive treatment like Multiple Blood transfusions Haemodialysis and assisted ventilation. The drug has virtually no side effects.

Notes

POSTER SESSION

PS - 1.1

CLONING, EXPRESSION AND PURIFICATION OF *P. FALCIPARUM* HGPRT

Rajendra S Rane, Jayashree Hariharan, K. Ayyanathan and Santanu Datta
Astra Biochemicals Pvt. Ltd., Sankey Road, Malleswaram, Bangalore, INDIA.

Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT) is a key enzyme of the purine salvage pathway in the malarial parasite which catalyzes the conversion of purines (Hypoxanthine, Guanine, Xanthine), in presence of Phosphoribosyl Pyrophosphate (PRPP), to their respective monophosphates. The HGPRT gene from *P. falciparum* has been used as a target for antimalarial drug development. This gene from a field isolate was amplified using RT-PCR and cloned into the pET8c vector and expressed in *E.coli* BL21(DE3) cells. The recombinant clone expressed the protein in a soluble form at levels of ~50%. The protein was purified to >95% homogeneity by FPLC using Mono Q Ion Exchange Chromatography. It was done in the presence of PRPP since the enzyme was found to totally lose its activity in the absence of PRPP. A continuous Spectrophotometric assay was developed where the reaction is monitored at the λ_{max} of the difference spectrum of the purine and its monophosphate. Using this assay the kinetic parameters of this recombinant enzyme were found to be comparable to the wild type enzyme for its substrates.

PS - 1.2

A COMPARATIVE STUDY ON THE MECHANISM OF ACTION OF HUMAN AND *PLASMODIUM FALCIPARUM* HGPRT

Dhiman Sarkar, Jayasree Hariharan, Rajendra S. Rane, Suresh M. Solapure and Santanu Datta
Astra Biochemicals Pvt. Ltd., Malleswaram, Bangalore,INDIA

Plasmodium falciparum causes malignant tertian malaria and is the most serious of all the forms of malaria. We have chosen the parasite Hypoxanthine-Guanine-Xanthine Phosphoribosyl transferase (PfHGPRT) as the target for rational drug design against this parasite. It is the key enzyme of the purine salvage pathway, the activity of which is crucial for the survival of the pathogen because of its incapability of *de novo* synthesis of purine nucleotides. PfHGPRT catalyses the removal of the pyrophosphate moiety from PRPP and attaches purine base to form respective nucleotides (IMP, GMP,XMP). For this study Human and Pf HGPRT were isolated from overexpressing *E.coli* clones harbouring recombinant Pf and human HGPRT genes (*E.coli* BL21DE3, Pet8c expression system). Our preliminary studies on the steady state kinetics of the forward reaction shows a clear difference between the human and parasite enzyme. For PfHGPRT, the Hanes-Wolf plot for xanthine or guanine gives a series of straight lines intersecting on the ordinate at different fixed concentrations of PRPP. When PRPP concentration is varied. The nature of plot obtained for different fixed concentrations of bases indicate a ping-pong mechanism with substrate inhibition. In contrast, control studies with human

HGPRT shows a ternory complex type mechanism further studies on product inhibition and isotope exchange between substrates are in progress. These results suggest a Ping-Pong type of mechanism for the PfHGPRT where PRPP binds first to the enzyme to release PPi and 5' -PR group remains attached with the enzyme. The base then binds th the functional group attached to the enzyme to form the nucleotide product.

PS - 1.3

CLONING, SEQUENCE DETERMINATION AND EXPRESSION OF THE *PLASMODIUM FALCIPARUM* DEAD-BOX RNA HELICASE.

K Saravana, K Patra, N Sahoo and F A S Kironde.
International Centre for Genetic Engineering and Biotechnology, Shaheed Jeet Singh Marg, New Delhi-110 067, India.
Vector Control Research Centre, Pondicherry, India.

Interspecies conserved proteins of *Plasmodium* may comprise molecules that are vital to structure and functional integrity of the parasite. Hence, these conserved protein could form the basis of intervention in the parasite life cycle. Here, we report the molecular cloning and determination of the sequence of complementary DNA (cDNA) which codes for the DEAD-box RNA helicase of *Plasmodium falciparum.* The cDNA sequence was obtained by reverse transcription and polymerase chain reaction (RT-PCR) using parasite messenger RNA and a set of primers designed on the basis of sequence data for previously described RNA helicases. The deduced protein sequence showed homology to other DEAD-box ATP-dependent RNA helicases, but also contained notable sequence differences when compared to bacterial, yeast, trypanosome, plant and human DEAD-box RNA helicases. The *P. falciparum* complementary deoxyribonucleic acid hybridized strongly to *P. yoelii* DNA suggesting that these two malaria parasite species contain significantly homologous DEAD-box RNA helicases. A 900-bp fragment of the *P. falciparum* gene was subcloned in expression vectors and expressed in *Escherichia coli.*

PS - 1.4

DNA POLYMERASES OF FILARIAL PARASITE *SETARIA CERVE* AND EFFECT OF POLYAMINES AND INHIBITORS.

J K Saxena, Utpal Pandya, and O P Shukla
Division of Biochemistry Central Drug Research Institute Lucknow 226 001.

DNA polymerases are the key enzymes in chromosomal duplication, the final step in nucleic acid biosynthesis pathways. In eurkaryotes DNA polymerase α, β, γ, δ and ε have been demonstrated and DNA polymerases α and δ are responsible for replication of SV 40 DNA. The DNA polymerase δ is responsible for leading strand synthesis whereas DNA polymerase α is involved in lagging strand synthesis. DNA polymerase β is a highly conserved enzyme in vertebrates which has role in gap filling DNA synthe-

sis and joining of Okazaki fragments during DNA replication. Polyamines viz., spermidine, spermine and diamine putrescine interact with nucleic acids and effect their structure and function. Targeting of drugs against specific enzymes and proteins of the parasites is one of the approach for combating parasitic diseases. We have therefore, studied enzymes involved in nucleic acid metabolism of filarial parasite *Setaria cervi* and its microfilarial stage in an attempt to detect differences between parasitic and mammalian enzymes. The polyamines viz., spermine and spermidine significantly activated the DNA polymerase activity of filarial parasite. DNA polymerase inhibitors showed inhibitory effect on the parasitic enzyme.

PS - 1.5

CLONING, SEQUENCE ANALYSIS AND EXPRESSION OF TRAP OF *PLASMODIUM CYNOMOLGI*

P S Sijwali, P Malhotra and V S Chauhan
International Centre for Genetic Engineering & Biotechnology (ICGEB), New Delhi 110 067, India

The thrombospondin related adhesive protein (TRAP) of *Plasmodium falciparum* is expressed by salivary gland sporozoites. Trap of *P. falciparum* and its homologue in *P. yoelii,* sporozoite surface protein-2 (PySSP-2), functions as a parasite receptor in the invasion of hepatocytes by sporozoites. Characterisation of an apparently functional protein like TRAP in a simian malaria parasite, *P. cynomolgi,* which closely resembles *P. vivax*, will be valuable in assessing its potential as a vaccine candidate. With this in mind we have cloned sequenced and expressed the TRAP gene from *P. cynomolgi bastianelli.*

The nearly full length TRAP gene was amplified from the genomic DNA of *P. cynomolgi* by PCR method. Sequencing of the gene revealed an ORF of 1635 bps with coding capacity for 545 amino acid residues. PcTRAP has an overall homology of 72% with PvTRAP including the presence of well-known conserved motif (WSPCTVTCGXGXRXR). The TRAP fragments, TRAP-a (aal-483), TRAP-b(aal-269) and TRAP-c (aa 270-483) were expressed in M15pREP-4 cells using pQE expression vectors, which will provide reagents to characterise TRAP.

PS - 2.1

USE OF GEOGRAPHIC INFORMATION SYSTEMS IN THE STUDY OF THE DISTRIBUTION OF MALARIA IN THAILAND

Kenneth J Linthicum, R Sithiprasasna, K Thimasarn, P Singhasivanon, K Nualchawee, D Dowreang, T Chongsuphajaisiddhi, S Suvannadabba, PL Rajbhandari and M Gomes
Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Centre for Malaria Region V, Department of Communicable Disease Control, Nonthaburi, Thailand; Natural Resources Program, Asian Institute of Technology, Bangkok, Thailand; Thailand Remote Sensing Centre, National Research Council of Thailand, Bangkok, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; and TDR, World Health Organization, Geneva, Switzerland.

Although malaria control programme efforts in Thailand have succeeded in reducing malaria morbidity, there continues to be fluctuations in malaria incidence. The 2 primary mosquito vectors in Thailand, *Anopheles dirus* and *An. minimus,* are associated with specific types of vegetation and are greatly influenced by environmental changes. Deforestation can result in the interruption of malaria transmission and there is some evidence to suggest that malaria transmission may become reestablished in areas where reforestation efforts have been successful. To better understand the temporal and spatial distribution of malaria in Thailand we analyzed transmission data with Geographic Information System (GIS). GIS software used in this study included Mapinfo, ARC/INFO and TNT Microimages Processing products. Digitized maps of provinces, districts, drainage systems, Contour lines, and land-use, normalized difference vegetation index data from NOAA Satellites and malaria incidence data between 1985-1995 were incorporated into a GIS. Malaria incidence varied between years in all districts and transmission was limited to borders with Burma on the west and Cambodia on the east where forests remain intact and cover more than 35% of the province. Thematic maps of malaria incidence landuse maps of Chantaburi Province in 1986, 1991 and 1996 as base maps were created and used to correlate malaria species and incidence with vegetation type. Timely and quantitative data on the occurrence of malaria, incorporated in a GIS database, can be used to anticipate change in incidence and help focus malaria control efforts in Thailand and other endemic regions.

PS - 2.2

FACTORS DETERMINING LOW AND HIGH INCIDENCE OF MALARIA IN APPARENTLY SIMILAR ECOTYPES IN NORTH DELHI

R C Dhiman, T Adak, R K Singh and V P Sharma
Malaria Research Centre (ICMR), 22 Sham Nath Marg Delhi-110 054, India.

Sant Nagar Burari area is a developing area in North-east Delhi and is endemic for malaria from where very high incidence of malaria was reported in 1993-94, while adjacent localities reported very low incidence. A study was therefore, undertaken in

Sant Nagar Burari area (high incidence, 4 subsites; pop. ca. 10000) and Mukundpur area (low incidence; pop. ca. 5000) incorporating ecological, entomological and demographic parameters to find out the factors responsible for the difference in endemicity levels.

Survey for prevalent anopheline breeding habitats and their potential larval density per dip, man hour density, (MHD) of adult mosquitoes was carried out fortnightly from late 1994 to 1996. Data on types of houses, family size, profession, migration history and presence/absence of cattle sheds were elicited through questionnaires.

It was found that ditches and underground cement tanks provided highest larval density in high endemic area as compared to ponds in low endemic area. Stagnant water in vacant residential plots showed highest frequency of positivity, 7 in 12 months against 3 in Mukundpur. MHD of *An. culicifacies* and *An. stephensi* was 53 and 6.75 Vs, 2.7 and 1.5 respectively in high and low endemic area. There was inverse relationship in the presence of cattle sheds and degree of endemicity (4.59% and 11.9% in high and low endemic areas respectively). The overall results of the study indicate that the duration of establishment of the localities is an important factor and microstratification of areas is essential for understanding the receptivity for malaria.

PS - 3.1

ESCAPING SITE OF *FASCIOLA GIGANTICA* CERCARIAE FROM REDIAE IN SNAIL

S C Yadav[1], S C Gupta[1], R L Sharma[1] and Satish Kumar[2]
1. Division of Parasitology, Indian Veterinary Research Institute, Izatnagar - U P 243 122 India.
2. National Biotechnological Centre, IVRI, Izatnagar.

Escaping site of *Fasciola gigantica* cercariae from developing rediae, inside laboratory bred and infected *Lymnea auricularia* was investigated using scanning electron microscopes (SEM). *F. gigantica* rediae obtained from infected snails were examined live for cercarial shedding pattern under stereoscopic microscope. These were also processed for electron microscopic scanning after fixing them in 25% gluteraldehyde in sodium cocodylate. The study revealed that there is no definite arrangement of germinal mass and cercariae were seen freely floating inside rediae with brisk movements. Interestingly, mature cercariae were observed emerging out of redial elastic and sticky tegument, leaving behind a self healing indentation. There was no definite site for liberation of the cercariae. We could not however, observe any birth pore for emergence of cercariae in the developing rediae.

PS - 3.2

CHARACTERIZATION OF FIELD ISOLATES OF *WUCHERERIA BANCROFTI* MICROFILARIAE

Usha Singh, Shailja Misra-Bhattacharya and R K Chatterjee
Division of Parasitology, Central Drug Research Institute, Lucknow-226 001, INDIA

There is no report on the existence of variable strains of *W. bancrofti* in India which may affect the success of chemotherapy, efficacy of vaccination or immunodiagnostics of human bancroftian filariasis in different geographical regions. Attempts were therefore, made in the present study to find out the existence, if any, of more than one strain of *W. bancrofti* in various filaria endemic regions of India. Microfilariae in night blood smears were collected from various states e.g. Maharashtra (Wardha); U.P. (Lucknow, Unnao and Ibrahimganj); Orissa (Bhubaneshwar and Rourkela); M.P. (Raipur); Andhra Pradesh (Kakinada, Viskhapatnam) and Kerala (Calicut). Morphological characterization of microfilariae was carried out by measuring total length, width and ratios of various fixed points. The latter included cephalic space (c.s.); nerve ring (n.r.); excretory pore (ex.p.); excretory cell (ex.c.); genital cell (G_1, G_{2-4}); anal pore (a.p.) and tail end (t.e.). Mf from Rourkela were the longest (351.66 µm + 4.76) while those from Raipur were shortest (221.82 µm + 3.95). Mf from Lucknow and Kakinada were closer in length while those from Bhubaneshwar, Unnao, Ibrahimganj and Calicut were closer in measurements. Mf from Wardha appeared quite different from all the other isolates. Besides this, the ratios of various fixed points also varied. Apart from morphological

characterization, immunological reactivity of serum antibodies of mf surfaces was also observed which revealed differential reactivity in IFAT.

The findings indicate the possibility of existence of more than one strain/subspecies of *W. bancrofti* in India. However, further studies like isoenzymic pattern and DNA characterization need to be carried out to substantiate the above results.

PS - 3.3

AN ULTRASTRUCTURAL STUDY ON THE ENCAPSULATION OF *PLASMODIUM VIVAX* IN THE MIDGUT OF A REFRACTORY STRAIN OF *ANOPHELES CULICIFACIES* SPECIES B

Nutan Nanda, T Adak, Sarbjit Kaur and V P Sharma
Malaria Research Centre (ICMR), 22-Sham Nath Marg, Delhi-110 054.

Successive stages involved during encapsulation of *P.vivax* in a refractory strain of *An. culicifacies* species B were studied using electron microscopy. Females of refractory and susceptible strains were fed on *P. vivax* - infected blood through artificial membrane and the midguts of the mosquitoes dissected at different time intervals after blood meal were processed for transmission electron microscopy. Electron dense multilamellar structures and vesicles were observed inside the midgut cells containing parasites. These structures were seen enveloping the degenerated ookinetes at later stage. Finally the parasite was found completely encapsulated by a thick melanin containing capsule. Haemocytes observed in close proximity to the encapsulated parasite probably contribute to capsule formation. In contrast, the parasite developed normally in the susceptible strain and the budding of sporozoites from sporoblast was observed within oocysts.

PS - 3.4

OBSERVATIONS ON THE GENITAL TRACT AND GAMETOGENESIS IN FEMALE *HAEMONCHUS CONTORTUS*

Manjeet Johal and Jatinderpal Singh
Department of Zoology, Punjabi University, Patiala - 147 002, INDIA.

Both ovaries project anterior, one slightly behind the other. These are thin walled tubes of simple squamous epithelium limited by a basal lamina and contain oogonia and oocytes at different stages of development. In the germinative zone, the oogonia having prominent nuclei and clear cytoplasm, divide mitotically and are arranged around a central enucleate tube like rachis which is not directly connected to the oogonia by their cytoplasmic extensions but sends out various branches in inter-oogonial spaces. In the growth zone of ovary, the primary and secondary oocytes attain a substantial increase in size, their cytoplasm becomes densely granular and nuclei possess prominent nucleoli and condensed chromatin. This region is tightly coiled around the intestine suggesting transmembrane flow of nutrients from gut to the gonads. The secondary hexagonal

shaped oocytes detach from the rachis and pass through the narrow oviduct and enter the seminal receptaculum, a modified part of the uterus, lined with oblique columnar epithelium whereas the rest of uterine wall is composed of cuboidal epithelial cells extending into various processes containing dense secretary granules which contribute to the formation of the outer uterine wall of the egg shell. Whole of the uterine tract shows spermatids which are more numerous in the seminal receptaculum. After fertilization the nuclear wall of the ovum breaks and meiotic division is completed. Simultaneously a vitelline membrane is laid around the ovum which is lifted up and a second layer of the egg shell, the chitinous layer, is formed endogenously under it, whereas the outermost layer in the form of a loose irregular envelop is secreted by the uterine epithelium. The ova lying in the proximal part of the uterus have already completed the first segmentation division before expulsion.

PS - 3.5

ANATOMICAL PECULIARITIES OF *COLUMBICOLA COLUMBAE* (PHTHIRAPTERA : ISCHNOCERA1) INFESTING PIGEON, *COLUMBA LIVIA* GMELIN

Adesh Kumar[1], S K Singh[2], Surman[2] and A K Saxena[2]
1. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Rishikesh (Dehradun), India.
2. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College Kotdwara (Pauri Garhwal), India.

Morphological features of digestive tract, reproductive organs, tracheal, nervous and circulatory systems of pigeon wing louse, *Columbicola columbae* Linn. have been worked out. The nature of oesophageal tube, crop, proventriculus, midgut caeca, ileum, rectal bulb, malpighian tubules and nephrosite has been observed. The numbers and distribution of stigmata and the disposition of various tracheal tubes have been noted. The nature of testicular follicles, vas deferens, vesicular apparatus, ejaculatory duct, polytrophic ovariole, lateral and median oviduct, vagina and spermatheca, has been recorded. The nature of heart and type of haemocyte have been studied. Lastly, the feature of cerebral, sub-oesophageal and three thoracic ganglia and the course of nerve have also been taken into consideration.

PS - 3.6

MORPHOLOGICAL VARIATIONS IN SOME ANOPHELINE MOSQUITOES INHABITING DIFFERENT ALTITUDES IN LOWER GARHWAL HIMALAYAS

C P Singh, R K Mahesh, B P Pokhriyal and R K Jauhari
Parasitology Laboratory, Department of Zoology, DAV (PG) College, DehraDun - 248 002 (UP), India.

The present investigation reveals the morphological variations in the wings/palpi in the

three recognised malaria vectors viz., An. fluviatilis, An. stephensi and An. culicifacies collected at different altitudes in lower Garhwal Himalayas. The finding have also been compared with the results of earlier works.Morphological variations among some of India Anophelines from the type form were first recorded by Christophers observed on extra dark band on the palpi of the female An. fluviatilis. Subsequently, Subramanian and Nagendra (1955) recorded an extra dark band on the palpi An. subpictus and An. Pallidus. Bhatnagar et al. (1958) made studies on the hypomelanic form of An. pallidus and incomplete development of sixth wing vein in colonized specimens of An.stephensi. Rajgopal and Chakraborty (1960) found variations in the hindtarsi and wing of An. annularis while Wattal et al. in the same year recorded a number of morphological variations in twenty species of Indian anophelines from the reference collections of NICD.

Since then after a gap of about twenty three years more emphasis was provided on the morphological cariations among the anophelines and the contrubutions of the following viz., Dash et al. (1983), Reuben and Suguna (1983), Nagpal and Sharma (1983), Dash et al. (1988), Gunasekaran et al. (1990) and Tyagi et al. (1991), one note worthy. It was Subbarao et al. (1987), who indicated the existence of an intermediate form, besides the type form and designated them as ecological variants. Recently, Nagpal and Sharma (1995) provided comprehensive information about the morphological variations among the recorded species of anopheline mosquitoes.

PS - 4.1

EVALUATION OF STICK PENICILLINASE AND MICROTITRE PLATE PEROXIDASE EIA SYSTEMS FOR DETECTION OF FILARIAL ANTIBODIES AGAINST Bm mf ES ANTIGEN

R Alli, K K Devi, R Narayan, M V R Reddy and B C Harinath
Department of Biochemistry and J B Tropical Disease Research Centre, MGIMS, Sevagram, India, 442 102.

Filarial IgG antibodies in patients sera were detected by Stick penicillinase as well as microtitre plate peroxidase enzyme immunoassays using *Brugia malayi* microfilarial excretory - secretory antigens (Bm mf ES). Comparative analysis of both the assays showed the simple and qualitative stick penicillinase ELISA to be superior to microtitre plate peroxidase ELISA, since by using the former assay more number of filarial cases have been detected. Analysing the same set of sera, stick ELISA gave a sensitivity of 90% while the plate ELISA showed a sensitivity of 68% and a specificity of 95%. Stick penicillinase ELISA requires lesser amount (100 pg) of antigen coating per test and more diluted (1:300) sera compared to microtitre plate peroxidase ELISA requiring 25 ng of antigen per test and 100 fold diluted sera. Thus, stick ELISA using penicillinase is about 750 times sensitive than plate ELISA using peroxidase. Both the assay system have been found to be very useful to detect active filarial infection particularly in clinical filarial cases where microfilariae cannot be detected.

PS - 4.2

MONOCLONAL ANTIBODIES SPECIFIC TO PLASMODIAL LACTATE DEHYDROGENASE

Namita Chandra, Nuzhat A Kaushal and Deep C Kaushal
Central Drug Research Institute, Lucknow - 226 001.

Lactate dehydrogenase (LDH), terminal enzyme of the glycolytic pathway, has been demonstrated in several species of malaria parasites and has been shown to play a role in energy metabolism of these parasites. Earlier studies from our laboratory have shown structural and immunochemical differences between the parasite and host LDHs. In the present study efforts were focussed on the purification and characterization of anti-LDH monoclonal antibodies, 25 were found to be specific to malaria parasite LDH as shown by the high reactivities of these monoclonals with parasite LDH and not with host enzyme. The effect of these monoclonal antibodies on the enzyme activity of parasite LDH was studied. Two monoclonals did not have any effect on the activity of parasite LDH while all other monoclonal antibodies were found to inhibit the parasite enzyme. Two monoclonal antibodies, one inhibitory type and other non-inhibitory type, were selected and characterized. The two monoclonals are directed against different epitopes as revealed by competitive ELISA studies. These monoclonal antibodies showed high reactivity with LDH from other species of malaria parasites and did not cross-react

with enzyme from other sources. These findings suggest that the target epitopes of these monoclonals are specific to malaria.

PS - 4.3

CHARACTERIZATION OF ANTI-IDIOTYPIC ANTIBODIES TO *PLASMODIUM* SPECIFIC ANTI-LDH ANTIBODIES

Neetu Tyagi, A Kaushal and Deep C Kaushal
Central Drug Research Institute, Lucknow - 226 001.

Malaria remains a major problem in tropical and subtropical countries. Considerable efforts have been directed towards control, prophylaxis and eventual eradication of malaria, however, the effective control of malaria will ultimately depend on rapid and accurate diagnosis. In view of the problems associated with parasitological diagnosis, emphasis has been given to immunological procedures and detection of malaria antigen appears to be a better approach for diagnosing malaria. In our laboratory, a sensitive and specific immunodiagnostic test, based on detection of parasite lactate dehydrogenase (LDH), has been developed employing polyclonal anti-LDH antibodies. The efforts to replace polyclonal with monoclonal were not very successful as most of the monoclonals generated against parasite LDH were inhibiting the LDH activity and could not be used in the immunodot test. The use of anti-idiotypic antibodies for obtaining non-inhibitory type anti-LDH antibodies is another approach. In the present study, polyclonal anti-idiotypic antibodies were generated in rabbits against the anti-LDH antibodies. The rabbits were immunized with affinity purified anti-plasmodial LDH IgG. The inhibition ELISA studies with immune rabbit sera revealed that the anti-idiotypic antibodies inhibited specifically the binding of anti-LDH antibodies to parasite LDH. These findings indicated the presence of anti-idiotypic antibodies in immune rabbit sera.

PS - 4.4

CHARACTERIZATION OF PURIFIED *SETARIA CERVI* ADULT ANTIGEN FOR FILARIA DIAGNOSIS

Neelu Srivastava, Deep C Kaushal and Nuzhat A Kaushal
Central Drug Research Institute, Lucknow 226 001.

Filariasis is a chronic deabiliating disease affecting millions of people worldwide. Due to insensitivity of parasitological method immunological procedures were adopted for diagnosing filarial infections. Antigens from heterologous filarial parasites have been used for filaria diagnosis in view of the non-availability of the human filarial parasite antigen in sufficient quantities. However, the use of crude antigenic preparation for diagnostic purpose, results in false positive reactions. This cross-reactivity may be due to the presence of phosphorylcholine (PC) and carbohydrate epitopes. IgG4 antibodies to these epitopes are generally absent in serum from filarial patients. In our previous

study, we have purified the *Setaria cervi* adult antigen employing different chromatography techniques. In the present study, we have characterized the purified antigen fraction (DFP-IV) and examined the IgG4 antibodies response in the patient sera by ELISA. SDS-PAGE and crossed immunoelectrophoresis analysis showed the presence of 2 major and 4 minor antigens in DFP-IV. This purified fraction gave least cross-reactivity with antibodies against other helminth parasites. The seta from microfilariae positive group contained IgG as well as IgG4 antibodies to DFP-IV, however, symptomatic cases had insignificant level of IgG4 antibodies to DFP-IV. These studies have also shown that IgG4 subclass antibodies were more specific than the IgG antibody assay with regard to endemic and non-endemic control sera. Thus, the measurement of IgG4 antibodies to S. cervi antigens appears to be a significant step toward improved serodiagnosis of filariasis.

PS - 4.5

DIAGONOSTIC POTENTIAL OF *SETARIA CERVI* EXCRETORY-SECRETORY ANTIGENS

Huma Mustafa, Deep C Kaushal and Nuzhat A Kaushal
Central Drug Research Institute, Lucknow - 226 001.

Excretory-secretory antigens of filarial parasites are know to be less complex in nature and more specific in defining infection as compared to somatic antigens. Due to the difficulties in obtaining sufficient antigenic material from human filarial parasite, antigens from *Setaria cervi,* a bovine filarial parasite have been used in the present study. The E-S antigens were isolated from the adult *S. cervi* worms by maintaining them in aseptic medium for 32 hr at 37°C and polyclonal antibodies were raised against them in rabbits. The E-S products showed the presence of 12-14 protein bands in the molecular weight range of 10-200 kD by SDS-polyacrylamide gel electrophoretic analysis. The polyvalent immune rabbit serum raised against them gave high titre value in the range of 1 : 120 000 to 1 : 250 000 and recognized 10-12 antigens in *S. cervi* E-S products. The E-S products were tested for the presence of host proteins using anti-BSA serum. 3-4 host components were found and one of them was identified as albumin. The potential of rabbit anti-E-S serum for the detection of filarial circulating antigen in patient sera was tested by Counter current immunoelectrophoresis. Initially, the rabbit anti-E-S serum gave non-specific reaction with the normal human serum, this may be due to the presence of host serum proteins. But when the immune rabbit serum was absorbed on BSA-sepharose beads, no cross-reactivity was observed with normal human serum. Therefore, the anti-E-S serum has sucessfully been used for the detection of circulating antigen using a panel of filarial patient sera.

PS - 4.6

SERODIAGNOSTIC STUDIES ON EXPERIMENTAL PARAMPHISTO MIASIS IN SHEEP AND GOATS

A Kaur, Urvashi, and H S Bali
Department of Veterinary Parasitology, Punjab Agricultural University, Ludhiana.

Hyperimmune serum against *Paramphistomum cervi* somatic antigen was successfully raised in rabbits. The antibodies in hyperimmune serum were readily detectable by agar gel precipitation test (AGPT). However, antibodies against this antigen in the serum of experimental lambs and kids were not detectable by AGPT.

None of the serum samples from immunized and control animals was found positive by immunoelectrophoresis (IEP). However, with hyperimmune serum raised in rabbits against *Paramphistomum cervi* antigen showed four clear lines of precipitation viz., albumin, alpha, beta and gamma globulins.

In lambs immunized with 3 Kr irradiated, metacercariae, precipitin lines were observed at 15 and 30 days post immunization and 15 and 30 days post challenge. Lambs immunized with 4 Kr, metacercariae precipitin lines were present at 30 days post immunization and at 15 and 30 days post challenge. Kids immunized at 3 Kr showed precipitin lines at 30 days post immunization and 15 and 30 days post challenge. The 4 Kr immunized group of kids showed precipitin lines at 30 days post challenge. Control groups of both lambs and kids did not give a positive reaction in CIEP.

A delayed cutaneous hypersensitivity reaction of significant intensity was observed in the immunized and control groups of both lambs and kids.

PS - 4.7

ANTIBODIES AGAINST DIFFERENT DOMAINS OF P0, A RIBOSOMAL PHOSPHOPROTEIN, INHIBIT *PLASMODIUM FALCIPARUM* GROWTH *IN VITRO.*

Sanchita Chatterjee, Subhash Singh and Shobhona Sharma.
Molecular Biology Unit, Tata Institue of Fundamental Research, Homi Bhabha Road, Bombay, India.

A differential immunoscreen using immune and patient sera detected antibodies against amino-terminal of P0, a ribosomal phosphoprotein homologue of *Plasmodium falciparum,* exclusively in the malaria immune persons. Different domains of P0 spanning the whole protein have been expressed as GST fusion proteins in *E. coli.* Polyclonal antibodies have been raised against these domains and they do recognize the 38kDa P0 protein from parasite extract on Western blot. About 10-15% patients suffering from Systemic Lupus erythrematosus (SLE) an autoimmune disease also possess antibodies

against P0. Such SLE patients sera, having high titre anti-P0 antibodies, recognize only the carboxy-terminal domain but not the aminoterminal domain. Polyclonal sera against both the aminoterminal and the carboxy-terminal domains of P0, and also the SLE patients sera inhibit parasite growth *in vitro*.

PS - 4.8

IN VITRO STUDY ON ANTISERA EFFECT TO TOLERANCE

Madhavi J & Susan Bhaskar Rao
Immunology Research unit, Dept. of Zoology, Kakatiya University, Warangal, A.P., India.

Specific antibody serves as one of the means of Tolerance induction. In the present in vitro study, - *Plasmodium gallinaceum* antisera was used to find its effect on Tolerance induction of *P. gallinaceum* antigen. Varying amounts of *P. gallinaceum* antisera were added to the normal spleen cell cultures and plaque forming cells were counted by adding homologous antigen *P. gallinaaceum* or heterologous antigen SRBC. The results showed that *P. gallinaceum* antisera inhibited *P.gallinaceum antigen* response by not SRBC response.

PS - 4.9

PROTECTION OF HAMSTERS AGAINST *LEISHMANIA DONOVANI* BY TWO NOVEL IMMUNOMODULATORS

Anju Puri, R Sahai, L M Tripathi, V M L Srivastava, W Haq[1], A Zaidi[2] and P Y Guru[2]
Department of Biochemistry, [1]Biopolymers and [2]Parasitology, Central Drug Research Institute, Lucknow 226 001, India

Structure activity relationship studies with N-acetyl-muramy l- L-alanyl-D-isoglutamine (MDP) have led to the identification of a number of derivatives with promising immunostimulant activity. Compound 86/448 and 89/729, two structural analogues of MDP, were found to provide significant degree of immuno-stimulation in mouse and guinea pig models. Oral administration of these peptides for two weeks provided protection in hamsters against a challenge infection with *Leishmania donovani* amastigotes. Studies were, therefore, also conducted to find out the efficacy of these modulators as adjunct to chemotherapy in *Leishmania*. For this both peptides were administered with or without stibogluconate, after the establishment of *L.donovani* infection. Subcurative dose of stibogluconate (5 mg/kg) reduced the parasite burden by 24%. However, in combination with 86/448 or 89/729 the efficacy of the drug increased to 75% and 67% respectively. Further studies showed that both the peptides produce TH-1 type of response as they significantly enhanced the production of Interleukin-2 and interferon-r by splenocytes.

The study indicates that combination chemotherapy using such peptides may be effective in reducing the dose and hence, the toxicity of antileishmanial drugs.

PS - 4.10

CROSS PROTECTION AGAINST VISCERAL LEISHMANIASIS BY KILLED *LEISHMANIA MAJOR* +BCG IN INDIAN LANGURS

Preeti Sharma, A Misra, J K Srivastava, Anuradha, J C Katiyar, and S Naik*.
Division of Parasitology, CDRI, Lucknow, India.
Department of Immunology, SGPGI, Lucknow, India.

Autoclaved *Leishmania major* (ALM) along with BCG is under trial as candidate vaccine against cutaneous leishmaniasis. We evaluated this combination against *Leishmania donovani* in the Indian Langur (*Presbytis entellus*) model we have developed. In an initial trial, we had observed that the monkeys vaccinated with single dose of intradermal (i.d.) ALM+BCG (3+3mg) were found almost devoid of any parasite after 6 months of the challenge, though, initially they did develop infection by day 50 post challenge (p.c.). Encouraged with this observation in another experiment, the monkeys were administered with three split doses of ALM+BCG (1+1mg) i.d., and were challenged with *L. donovani* amastigotes. On day 50 p.c. three of the four vaccinated animals were found devoid of any parasite but the fourth one developed infection (125/500) which also resolved by daay 150 p.c.. In comparison, the parasite burden in infected control group, was to the tune of 108-775/500 cell nuclei. The status of vaccinated group remained the same till day 230 p.c. when the experiment was to the tune of 108-775/500 cell nuclei. The status of vaccinated group remained the same till day 230 p.c. when the experiment was terminated. Prior to challenge, there was an initial rise in antileishmanial antibodies in vaccinated monkeys as compared to unvaccinated group but after the challenge the antibody levels in unvaccinated monkeys were higher. There was no demonstrable antigen-specific response in vaccinated monkeys to either ALM or autoclaved *L. donovani*. It appears that ALM+BCG provides cross protection against *L. donovani* and in contrast to single vaccination, triple dose is more efficient.

PS - 4.11

ANTIBODIES TO A NON-REPEAT SEQUENCES OF *PLASMODIUM FALCIPARUM* ANTIGEN Pf155/RESA PREVENT CYTOADHERENCE AND MEROZOITE INVASION *IN VITRO*

A B Siddique, N Ahlborg, B Wahlin Flyg, P Perlmann and K Berzins.
Department of Immunology, Stockholm University, S-106 91 Stockholm, Sweden

In *Plasmodium falciparum* malaria, antigens derived from surface of infected erythrocytes or parasites play important roles in either the cytoadherence or merozoite invasion process. It has previously been shown the non-repeat region of antigen Pf155/RESA contains a hexapeptide motif being highly homologous to a cytoadherence related se-

quence of erythrocyte band 3. We now report antibodies to multiple antigenic peptides (MAPs) containing this homologous hexapeptide motif from either Pf155/RESA or band 3 inhibit the binding of *P. falciparum* infected erythrocytes (Palo Alto strain) to melanoma cells *in vitro*. Furthermore, rabbits immunized with these MAPs generated antibodies showing specific reactivity with band 3 and Pf155/RESA respectively, in immunoblotting and immunofluoresence, and the antibodies also inhibited cytoadherence of *P. falciparum* infected erythrocytes of melanoma cells *in vitro*. While antibodies to the hexapeptide motif did not interfere with parasite growth *in vitro*, antibodies to neighbouring sequences (residues 199-214) inhibited merozoite invasion of both $RESA^+$ amd $RESA^-$ parasite *in vitro*. Our results indicate that antibodies to epitopes formed by these non-repeat sequences of Pf155/RESA may mediate parasite neutralization and cytoadherence inhibition, and thus, the immunogens based on these sequences are of interest for development of *P. falciparum* malaria vaccines.

PS - 4.12

IgE IN *PLASMODIUM FALCIPARUM* MALARIA, A TH2-TYPE OF IMMUNE RESPONSE AND ITS POSSIBLE PATHOGENIC ROLE

Gehad ElGhazali[1,2], Hedvig Perlmann[2], ASM Rutta, Peter Perlmann[2] and Marita Troye Blomberg[3]

1. Faculty of Medicine, University of Khartoum, Sudan.
2. Department of Immunology, Stockholm University, 106 91 Stockholm, Sweden.
3. National Institute for Medical Research, Amani, Tanzania.

People living in *Plasmodium falciparum* endemic areas frequently have elevated levels of total as well as *P. falciparum* specific serum IgE. Elevated IgE levels suggest an immunoglobulin switch from IgM caused by the "switch factors" IL-4 and IL-13 released by activated Th2-cells. To investigate the role of Th1 and Th2 cells in the human *P. falciparum* system we used the ELISPOT assay to determine the ratio of IFN-γ and IL-4 producing cells after specific antigen or mitogen activation *in vitro*.The donors were individuals who had acquired immunity through natural exposure to the malaria parasite, *P. falciparum*. The anti-malarial IgE levels in serum were correlated with an increased ratio of IL-4/IFN-γ producing cells. Thus, donors with ratios of IL-4/IFN-γ > 1 exhibit mean serum anti-malaria IgE levels significantly greater than those with ratios < 1. Our data suggest a skewing in the balance of $CD4^+$ T-cells towards the T helper 2 subset in naturally *P. falciparum* primed individuals, associated with elevated serum levels of anti *P. falciparum* IgE levels. Notwithstanding its potential protective capacity, IgE very likely also contributes to the pathogenesis of *P. falciparum*. This is supported by the finding that IgE elevation is consistently highest in cerebral malaria. IgE containing immune complexes may give rise to local over-production of TNF by monocytes through cross-linking of CD23, the low-affinity receptor for IgE. This is supported by 1) the *in vitro* induction of TNF in adherent blood mononuclear cells from normal donors after exposure to IgE containing malaria sera in the presence of anti-IgE antibodies and 2) the positive correlation between IgE and TNF levels in patients with

cerebral malaria. Thus, IgE and the underlying skewing of the Th2/Th1 balance are contributing to the pathogensis of malaria.

PS - 4.13

PROTECTIVE POTENTIAL OF HEAT KILLED *LEISHMANIA* PROMASTIGOTES + BCG IN EXPERIMENTAL KALA-AZAR

J K Srivastava, Anuradha, P Sharma, and J C Katiyar,
Division of Parasitology, Central Drug Research Institute, Lucknow- 226 001, India.

The outbreak of visceral leishmaniasis or kala-azar is of considerable public health concern. The disease is highly prevalent in Bihar, resulting in high mortality every year. Resistance of parasites against Sodium Stibo Gluconate (SSG) and Pentamidine are becoming common feature. Therefore, development of immunoprophylactic(s) seems to be the only effective tool for controlling the disease. Autoclaved *Leishmania major* (ALM), a vaccine developed against cutaneous leishmaniasis was provided by WHO with recommendations to evaluate its potential against visceral leishmaniasis.

In the present investigation autoclaved *Leishmania major* (ALM) and autoclaved *Leishmania donovani* (ALD) preparations were evaluated in hamsters against a challenge of *Leishmania donovani* infection. Groups of hamsters were intradermally immunized with ALM or ALD (1mg protein each) either alone or in combination with BCG (human dose) followed by two boosters of equal amount at the interval of 15 days. On day 15 of the last booster, the vaccinated and unvaccinated control groups were challenged with 20 million promastigotes intracardially. Splenic biopsy done after day 45 post challenge revealed almost complete protection in about 50% animals of ALM+BCG vaccinated group and rest of the animals had very low parasite burden. In a cumulative manner ALM+BCG registered 93% parasite suppression. The prophylactic efficacy in ALD+BCG vaccinated group was to the tune of 89% followed by ALD (79%), ALM (56%) and BCG (29%).

The study corroborates the findings in Indian langur model vaccinated with ALM+BCG and warrants extensive validation of this combination including the trials in human kala-azar.

PS - 4.14

IDENTIFICATION OF T CELL AND B CELL EPITOPES IN THE NON-REPEAT AND REPEAT REGIONS OF *PLASMODIUM FALCIPARUM* LSA-1 PROTEIN

Sunil K Joshi, A Bharadwaj, S Chaterjee and V S Chauhan
Malaria Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110 067, India.

We report here the presence of new T_H cell and B cell epitopes in the non-repeat and repeat sequences of *P. falciparum* LSA-1 protein. This is based synthetic peptides in the experimental *P. berghei* ANKA-mouse model. We have tested eight synthetic peptides and found that the 19 amino-acid long peptide LSA-1.2 is a dominant T cell epitope, since it triggers *P. berghei* immune splenocytes of C57BL/6 (H-2^b), BALB/c (H-2^d) mice and Thamnomys (natural host of *P. berghei* ANKA) to proliferate. The peptide LSA-1.2 probably triggers a T_{H1} type of response, as proliferating T cells. On looking for overlapping B cell epitopes in the potential T cell epitope sites, anti-sporozoite antibodies show reactivity to LSA-1.1 and LSA-1R. A repeat based peptide LSA-1R stimulates T cell proliferation only in immune BALB/c spleen cells. We show that not only do these LSA 1.2 and LSA-1R sequence trigger T cell activity in parasite immunised animals, but these sequences on their own can generate memory T cells on immunization which are later expanded on secondary stimulation with the homologous peptides. Thus, our study establishes the presence of two T cell epitopes and two B cell epitopes in *P. falciparum* LSA-1 protein. LSA-1.2 peptide deserves special attention since it is a dominant T cell epitode triggering proliferative responses in two different mice haplotypes (H-2^d and H-2^b) as well as in the natural host of the parasite; the Thamnonys. The detailed results of this study will be discussed.

PS - 4.15

NATURALLY OCCURRING *PLASMODIUM FALCIPARUM* SPECIFIC IMMUNE RESPONSES IN AN ENDEMIC ZONE OF ANDHRA PRADESH, INDIA

P L Prakash Rao[2], and J D Goud[1]

1. School of Biotechnology, JNT University, Mahaveer Marg, Hyderabad -28.
2. Malaria Group, International centre for Genetic Engineering & Biotechnology, Aruna Asaf Ali Marg, New Delhi-110 067.

Delineation of naturally occurring immune responses to malaria antigens in relation to clinical, parasitological and transmission levels besides providing useful epidemiological information also helps to establish necessary infrastructure and to evolve logistics for carrying out detailed field trails of newer control strategies such as vaccines, bednets or launching of new drugs.

In a cross-sectional study, blood samples collected at different time points (pre, post and during transmission) from individuals belonging to an endemic area of Andhra Pradesh over a period of time were tested for plasmodium antigen specific immunoglobulins by indirect ELISA using a soluble extract of *Plasmodium falciparum*. The results showed that large proportion of samples were seropositive in different time periods and age groups (55.7% in < 5 yrs to 78.1% in > 40 yrs) with peaks during monsoon periods. The prevalence and titres of antibody were greater in adults than in children ($p < 0.01$). The antibody levels measured in O.D. at different time periods and GMRT values showed significant fluctuations, increasing during transmission periods and decreasing sharply during non-transmission periods (Wilcoxon rank sums test, $p < 0.05$). There was no

significant difference in specific antibodies between males, females and parasitaemic and non-parasitaemic individuals ($p>0.05$) however, the antibody levels increased with increasing number of episodes experienced at the time of sample collection ($p < 0.01$). The results demonstrate that the levels of malaria endemicity affects both the prevalence and the concentration of antibodies and the rate of acquisition of immunity with age.

PS - 4.16

HUMORAL IMMUNE RESPONSES IN EXPERIMENTAL ASCARIDIASIS

S S Lal, M V Subba Rao, Savita Sharma and D Kumar
Department of Zoology C.C.S. University, Meerut - 250 005.

The most costly parasites in terms of production losses are the gastro-intestinal nematode parasites in ruminants and poultry. Present studies pertain to humoral immune responses in gastro-intestinal disease ascaridiasis due to *Ascaridia galli* in WLH chicks. The WLH chicks were first immunized with adult somatic, metabolic, larval somatic and egg antigens and then challenged with 100 pure embryonated eggs of *A. galli*. Immune responses in terms of antibody titres were studied from different experiment groups through HC-partition plates. Agarose gel electrophoresis revealed albumin, alpha-1 globulin, alpha-2 globulin, Beta globulin and gamma globulin depicting immune responses. Immunogenecity of different lyophilized antigens were observed.

Group-I (Control): After first week IgG level was 4.32 gm/L. In post immunization IgG was elevated with overall rise from 4.32 gm/L to 6.14 gm/L. Overall rise of IgA was from 2.09 gm/L to 3.11 gm/L. IgM antibodies showed significant increase at fourth week. Group-II :- Immunized with adult somatic antigens. IgG increased significantly (P 0.005, ANOVA TWO WAY) by second week. Both IgA and IgM antibodies revealed significant increase after first and second week respectively. IgM showed overall declination from 3.65 gm/L to 2.29 gm/L. Group III: - Immunised with metabolic E/S antigens. IgG antibodies revealed significant increase with overall elevation from 4.8 gm/L to 10.35 gm/L. IgA antibodies were elevated significantly after one week but they decreased in third and fourth week. IgM antibodies revealed overall declination from 2.83 gm/L to 1.77 gm/ L. Group-IV:-Immunized with larval somatic antigens. IgG revealed overall rise from 6.29 gm/L to 16.29 gm/L. IgA showed declination. IgM revealed overall fall. Group -V:-Immunized with egg antigens. IgG showed overall rise from 5.42 gm/L to 12.19 gm/L. IgA revealed significant fall. IgM also revealed declination from 3.25 gm/L to 1.86 gm/L.

PS - 4.17

EFFICACY OF INTEGRAL MEMBRANE PROTEINS AGAINST *L. DONOVANI* INFECTION IN SUSCEPTIBLE GOLDEN HAMSTERS

Ujjal K Singha, Sangeeta kulkarni, Vahab Ali, A K Rastogi and Vinod Bhakuni

Division of Membrane Biology and Biochemistry#, CDRI, Lucknow, India.

Chemotherapeutic agents in use against human visceral leishmaniasis when used as required may exert severe toxic reactions in treated individuals; furthermore they often fail to produce complete cure. Patients become unrespoive to conventional doses of antimonials, usually after repeated treatment in replapses. Some patients respond to higher doses of antimonials, but toxicity with high doses usually precludes an adequate duration of such therapy. To circumvent some of these problems, a number of investigators are examining the avenues for immunological intervention.

Since individuals recovered from leishmaniasis are resistant to reinfection, vaccination may be the best answer to the control of the disease. As an initial step towards this aim, we have extracted the membrane antigens of *L. donovani* promastigotes using Triton X-114 detergent and also purified some of them. Susceptible golden hamsters were vaccinated using these mixture of antigens and purified antigens and their liposomal preparations. The schedule and dose tried were 50µg followed by 25µg each two times within a gap of 2 weeks each. The hamsters were finally infected with promastigotes 2 weeks after the final booster.

PS - 4.18

IMMUNOPROPHYLACTIC/VACCIN POTENTIAL OF LIPOSOMISED LIPOPHOSPHO GLYCAN OF *LEISHMANIA* PROMASTIGOTE AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS

Vahab Ali, U K Singha and V. Bhakuni
Division of membrane Biology, Central Drug Research institute, Lucknow

Visceral leishmaniasis (kala-azar) is a fatal disease if not attended properly at an early onset of the disease. In recent past the reports of unresponsiveness of known antimonial i.e. sodium stibogluconate and pentamidine has created deep concern towards the control of the disease. In addition to conventional therapy, immunoprophylactic agents need serious consideration. So far, no vaccine for visceral leishmaniasis is available. The resistant to reinfection in treated kala-azar patients suggests that vaccination against visceral leishmaniasis is possible. One of the key determinants of parasite infectivity and survival in the host macrophages is a novel cell surface molecule, lipophoglycan (LPG). LPG is a heterogeneous lipid containing polysaccharide aand is produced by all *Leishmania* species. It plays an important role in the biology of parasite because of its surface location, its developmental regulation during the life cycle and the reduced virulence of the LPG-deficient organisms.

We have isolated and purified the LPG from promastigotes of non-pathogenic strain (UR6) of *Leishmania donovani* and studied the potential of this LPG in providing protection against experimental visceral leishmaniasis. Susceptible gloden hamsters were vaccinated with purified LPG and incorporated in different types of liposomes. The

liposomes are strong immunopotentiators for various antigens, and being biodegradable, non-toxic and may be used as an alternative adjuvant for human. Production of antibody level in diseased and protective animals was measured by ELISA. Studies in susceptible golden hamster demonstrated that pretreatment with LPG from 10-28 days prior infection provides significant protection in hamster against *L.donovani* infection. This protective efficacy of LPG against *L. donovani* infection in hamsters was found to be significantly enhanced by its incorporation in cholesterol containing unilamellar vesicles.

PS - 4.19

ISOLATION, PURIFICATION AND IDENTIFICATION OF LOW MOLECULAR WEIGHT INTEGRAL MEMBRANE PROTEINS OF *LEISHMANIA DONOVANI* PROMASTIGOTES

Sangeeta Kulkarni and Vinod Bhakuni
Division of Membrane Biology, Central Drug Research Institute, Lucknow 226 001

Cell mediated immune (CMI) responses in leishmaniasis are very low as the parasite in its intracellular form resides and proliferates in macrophages- the very cell of the immune system whose sole function is curtail the spread of parasitic pathogens. Hence immune intervention in conjunction with the conventional chemotherapy and/or immunotherapy seems to be the need of hour. Parasite surface molecules namely (viz.) glycoprotein gp63, lipophosphoglycan and integral membrane proteins are of special interest in this connection. Since it is the parasite surface that first encounters the host immune system, the interplay between the two is most likely to exert a profound influence over the outcome of the disease. IMPs being amphipathic in nature are most efficiently extracted by a detergent, since detergent being amphiphilous themselves satisfy both the hydrophilic as well as the lipophilic passions of the membrane proteins. Isolation of IMPs with the non-ionic detergent Tx-114 (Triton X) is a temperature dependent phase partition method. The *Leishmania donovani* strain UR-6 promastigotes are extracted with the detergent at 0°C by mild sonication followed by phase partition on a sucrose cushion at 37°C. Detergent enriched layer containing all the putative IMPs was freed of the bulk of unassociated detergent on an amberlite XAD-2 column. Finally individual proteins were purified by either ConA lectin affinity chromatography or polyethylene glycol precipitation. ConA-sepharose column separates out glycoproteins that have either α- mannose or α-glucose as its terminal sugar moiety. A single glycoprotein of MW 30 kDa was obtained. The detergent layer was subjected to 6% and 12% PEG precipitation. A single protein band that was less than 14 kDa were observed in each step. Prophylactic studies in golden hamsters with these proteins are in progress.

PS - 4.20

STUDIES ON MOSQUITO DEFENSE SYSTEM AGAINST A BIOCIDE, *BACILLUS SPHAERICUS*

Lalitha Kabilan[1], S Vani[2], T R Rao[3] and Pitchappan[2]
1. Malaria Research Centre, 22-Sham Nath Marg, Delhi - 110 054.
2. Madurai Kamraj University, Madurai (T N)
3. Centre for Research in Medical Entomology, Madurai (T N)

Insects display a variety of defense mechanisms to protect themselves against invading pathogens. Mosquitoes are susceptible to a number of microbacterial infections, which raised the possibility of developing bacteriological agents (biocide) as a mosquito control measure. *Bacillus sphaericus*(Bs) is a potential biocide against mosquito larva. However, different species of mosquito larvae differ in their susceptibility to this biocide suggesting the role of host factor in susceptibility. We have compared the protein profile of two species of mosquito larvae differing in their susceptibility to comparing the protein pattern in Bs-exposed and control (Bs-non exposed) larvae, using SDS-Poly Acrylamide gel electrophoresis. Comparison of proteins of uninfected larvae from both the species revealed that a number of proteins are common to both the 86kDa, 69kDa and 52kDa are present in *Aedes* larvae and not in *Cluex* larvae. Comparison of proteins of infected larvae of *Culex* and *Aedes* have shown that some proteins of 52 to 34kDa; 83 and 87kDa are present in *Aedes* larvae and *Culex* larvae respectively. But certain proteins which have been shared by uninfected larvae of both the species disappeared in the infected *Culex* larvae and not in *Aedes* larvae. The biological significance of these proteins in the antibacterial activity need to be assessed.

PS - 4.21

EFFECT OF POTENTIAL ANTI-RELAPSE ANTIMALARIA COMPOUND CDRI 80/53 ON IMMUNE RESPONSES IN SWISS MICE.

Neelu Gupta, Anju Puri, Ragini Srivastava and S K Puri
Divisions of Microbiology and Biochemistry, Central Drug Research Institute, Lucknow.

Compound CDRI 80/53, an enamine analog of primaquine is being developed at this Institute as a potential anti-relapse antimalarial drug. This compound would require daily administration in 5-14 dose regimens for treatment of *P. vivax* relapses. In addition, in view of the relative safety of CDRI 80/53 over primaquine, the new compound is an ideal candidate for prophylaxis against malaria which would require long term daily administration during the transmission season in endemic areas. The present study was carried out to compare the effect of CDRI 80/53 and primaquine on cellular immune responses (lymphocyte proliferation, macrophage migration and DTH) and humoral immune responses (plaque forming cell assay, haemagglutination assay) in normal Swiss mice. Groups of mice were administered primaquine (8 mg/kg) or CDRI 80/53 (10 mg/ kg), orally once daily for 28 days. Results showed that both CDRI 80/53 and

primaquine marginally enhanced the Con-A induced proliferative responses of lymphocytes. There was no alteration in macrophage migration indices or DTH response to sheep erythrocytes with both the drugs. The humoral immune responses as indicated by plaque forming cell counts as well as haemagglutination assay with sheep erythrocytes were also not significantly altered as compared to the control group. The study provides evidence that long term administration with CDRI 80/53 does not produce clinically undesirable immunosuppression in experimental animals.

PS - 4.22

VARIATION IN SERUM IFN-γ AND URINE CORTISOL DURING *PLASMODIUM BERGHEI* INFECTION IN MICE.

A Rodriguez-Acosta[1], M Pulido-Mendez[2], M E Giron[1], I Aguilar[1] and H Finol[3].
1. Universidad Central de Venezuela. Tropical Medicine Institute[1].
2. Experimental Medicine Institute.
3. Centre of Electron Microscopy, Science Faculty[3] . P.O.Box : Apartado 47423 Caracas 1041 - Venezuela.

Some clinical manifestations of severe malaria are not fully understood. These include in certain patients the rapid development of cardiovascular collapse, shock, hypothermia and other phenomena that could be related with diminished supportive action of glucocorticoids. Although there is not a link close correlation with pathological changes found in the human disease, *P. berghei* infection in mice produces a severe and fatal illness that in various aspects resembles *P.falciparum* malaria and it remains as a useful model in malaria research. In this work we described the associated changes in urine cortisol and IFN-r in the course of *P.berghei* infection, as an approach to the understanding of pathogenic mechanisms in this disease. C57B1/6 mice were inoculated with $1x10^6$ *P. berghei* parasitized erythrocytes. Pool of serum samples and pool of 24 hours urines were collected in groups of animals at 0, 3, 6, 9 and 12 days post-infection. IFN-r and urinary cortisol were determined by ELISA assay and conventional techniques, respectively. The results showed a profound depression of cortisol urinary excretion (0.027 µg/mouse/24 hours) that was evident at the third day post-infection, followed by a light increase that peaked at the sixth day (0.070 µg/mouse/24 hours) when serum IFN-r levels also were in a maximum (2130 pg/ml). There was a correlation between both measures (r=0.80) when levels were analyzed for the 3, 6 and 9 days. Our results suggest that a significant reduction in cortisol secretion can contribute to the pathophysiology of malaria. Recently, we have also reported adrenal gland ultrastructural damage. IFN-γ high levels can produce organ damages mediated by other cytokines such as TNF.

PS - 4.23

ANTIBODY RESPONSE TO PEPTIDE CONSTRUCTS REPRESENTING N-TERMINAL CONSERVED PORTION OF MSA-2 OF *PLASMODIUM FALCIPARUM*

Andrei A Lougovskoi, Ashima Bharadwaj, Pawan Sharma and Virander S Chauhan
International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067,
INDIA

The MSA-2 of *Plasmodium falciparum* is a 45-52 kDa membrane protein located on the surface of merozoite. Synthetic peptides representing conserved MSA-2 sequences have shown protection in mice and MSA-2 is being considered as a possible component of a blood stage malaria vaccine. We also found that MSA-2 sequence of Indian strains are closely related to FC-27 strain isolated in Papua New Guinea. The purpose of our study was to evaluate antibody response to N-terminal conserved portion of MSA-2 using synthetic peptides and to design a suitable construct in order to focus the antibody response to the B-epitope SNTFINNA shown to be involved in protection. The constructs used in this study, included the entire constant N-terminal region peptide, 23 residues long (P-23), its hybrid moleules obtained either by chemical ligation reaction or co-linear synthesis over tetanus toxoid T-helper epitope tt(830-844) and tetanus toxoid protein conjugates. The ability of peptide constructs to induce antibody response was tested in two mouse strains BALB/c and C57B1/6. Co-linear synthesis of SNTFINNA-epitope over 23 residues long peptide greatly enhanced the antibody response to the specific epitope compared to immunisation with P-23. Furthermore, this chimeric construct was also found to be highly immunogenic in Balb/c mouse strain; antibody response to the epitope SNTFINNA was comparable to that obtained upon SNTFINNA-tetanus toxoid protein conjugate immunisation. Neither ligation of p-23 to tt(830-844) T-helper epitope nor their physical mixture improved immunogenicity of this peptide. Peptide construct consisting of SNTFINNA-epitope synthesized over tt(830-844) T helper epitope was able to induce appreciable antibody response to this epitope only in C57B1/6 mouse strain. Possible implication of synthesized constructs as vaccine candidates are discussed.

PS - 4.24

IMMUNOBLOTT AND ELISA ANALYSIS OF E/S ANTIGENS OF *GASTROTHYLAX CRUMENIFER*

Md Khalid Saifullah, Gul Ahmad and W A Nizami
Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh-202 002 (U.P.), India.

The E/S substances released by *Gastrothylax crumenifer* during *in vitro* incubations were analyzed in SDS-Gradient (7-15%) Polyacrylamide Gel Electrophoresis (SDS-PAGE). Immunogenicity of E/S products was evaluated by Immunoblotting and En-

zyme Linked Immunosorbent Assay (ELISA) with sera raised against E/S proteins in laboratory reared rabbits. The electropherogram of the E/S products resolved a total of 38 polypeptides by Silver Staining having an apparent molecular weight ranging from <29 kD to > 205 kD.

In order to probe the antigenicity of metabolic products of *G. crumenifer* the fractionated E/S proteins by SDS-PAGE were transblotted on a nitrocellulose membrane and detected by rabbit anti E/S antisera. The analysis of results revealed a total number of 27 polypeptides, mostly of high molecular weight, which are recognized by the homologous primary sera. Analysis by ELISA revealed that a dilution of as high as 1 : 3200 could react with the E/S antigens. A cut off point of 1 : 3200 dilution was worked out beyond which homologous sera could not detect E/S antigens. Further, when the E/S antigens were allowed to react with anti whole homogenate hyperimmune sera it was observed that the titre of IgG increased upto a dilution of 1 : 12800.

Further studies to characterize the antigenic components are in progress using immunoaffinity chromatography.

PS - 4.25

AMASTIGOTE SPECIFIC ANTIGEN OF *LEISHMANIA DONOVANI*

Neehar Bhatia and Kamal K Kamboj
Division of Parasitology, Central Drug Research Institute,Lucknow - 226 001, India.

Leishmania donovani, the causative agent of visceral leishmaniasis or kala-azar is a dimorphic protozoan parasite existing as promastigote form in the sandfly vector and an amastigote form in the reticuloendothelial system of the vertebrate host. While the antigens associated with the promastigote forms have been characterized to a great extent, those of the amastigote stages are largely unknown

Balb/c mice were inoculated with 1 x 10^6 amastigote forms of *L. donovani* purified from the spleen of experimentally infected golden hamsters. Two months later when the mice were operated for spleen biopsy and L.d. bodies were detected in Giemsa stained smears. These mice were treated with subcurative doses of SAG (10 mg/kgx5 days). Three weeks later when the anti-amastigote antibody titre was 1:16000, the splenocytes of one mouse were fused with P3U1 mouse myeloma cells in the presence of 35% polythylene glycol. 642 hybrid colonies were detected and screened for the presence of parasite specific antibodies. Eleven clones were found to secrete anti-amastigote stage antibodies and were cloned by limiting dilution method using thymocytes as feeder cells. Ten of the moAbs did not show any reactivity with the promastigote stage of the parasite indicating their stage specificity. By western immunoblotting, six of these moAbs identified antigen of approximately 20 kD in extracts of amastigotes. These antibodies were found to have potential in detecting amastigotes in the tissue biopsies and histological preparations in an indirect immunofluorescence assay.

Stage specific antigens of amastigote stages of *L.dsonovani* might be important molecules for immunoprophylactic and immunodiagnostic studies.

PS - 4.26

MONOCLONAL ANTIBODIES AGAINST THE BLOOD STAGES OF *PLASMODIUM YOELII*

Roopali Chaturvedi and Kamal K Kamboj
Division of Parasitology, Central Drug Research Institute, Lucknow - 220 001, India.

Monoclonal antibodies were produced against the blood stages of *Plasmodium yoelii nigeriensis,* a rodent malaria parasite. Briefly, Balb/c mice were hyperimmunized with blood stages of this parasite by repeated challenges after subcurative chloroquine therapy. Splenocytes of mouse showing antibody titre of 1:8000 were fused with P3U1 mouse myeloma cells in the presence of 35% polyethylene glycol. Hybrid colonies were screened for parasite specific antibody by indirect immunofluorescent antibody assay employing air dried, acetone fixed parasitized erythrocytes and positive hybrids were cloned by limited dilution technique using mouse thymocytes as feeder layer. Eight positive clones were identified and these exhibited three different types of fluorescence patterns. Two moAbs reacted only with the mature schizonts inside the erythrocytes. Three moAbs reacted with the membrane of parasitized erythrocytes irrespective of the stage of the parasite. These did not show any reactivity with the internalized parasite. The remaining three moAbs gave irregular fluorescence pattern of large and small dots on the infected erythrocytes irrespective of the stage of the parasite. None of the moAbs showed any reactivity with the normal erythrocytes.

PS - 5.1

NOVEL WAY OF DEVELOPMENT OF AMPHISTOME CERCARIA

R K Prajapati and A K Maurya
Parasitology Laboratory, Department of Zoology (CAS), Banaras Hindu University, Varanasi - 221 005, INDIA.

In general cercariae develop within the body of mother sporocyst or redia and almost mature cercariae are released either through birth pore or by rupture of mother sporocyst/redia. But the development of present amphistome cercaria is rather unique in the sense, its development takes place in the host tissue (digestive gland of snail) rather than within the body of redia. When the snail, *Lymnaea luteola,* the host of present cercaria were dissected, a large number of developing cercariae were found in the digestive glands of the host. It was speculated that there could have been very few and large sized sporocyst/redia which might have been damaged during dissection. But even after very careful dissection of second, third, fourth and fifth snails the result remained the same. We did find small rediae full of germ balls but even the largest redia was much smaller than the cercaria, which ruled out any possibility of development of cercariae inside the body of redia and some rediae were observed releasing germ ball through birth pore and these germ balls can be traced to gradually metamorphose into cercariae. All these observations led to the conclusion that tiny rediae which must have been released from mother sporocyst, contain a few developing germ balls. These rediae grow in size, but still much smaller than the cercariae, release germ balls in the host tissue (digestive gland) where they grow and gradually metamorphose into cercariae.

PS - 5.2

N Srivastava
(Abstract not received)

PS - 5.3

COMPUTER BASED INDIAN ANOPHELINE LARVAL IDENTIFICATION KEY

B N Nagpal, Aruna Srivastava and Rekha Saxena
Malaria Research Centre 20, Madhuban, Delhi - 110 092.

Revised malaria control strategy inter-alia lays primary emphasis on bio-environmental methods to reduce receptivity. Bio-environmental control aims at source reduction, and therefore larval identification has become important in malaria control. Larval identification is rather difficult and requires an experienced specialist. To simplify this, computer based larval identification key of Indian Anophelines has been developed in Foxpro 2.5. The software developed can identify larvae rapidly and the module can also be used for in this technique a user has to follow the morphology of the larvae and enter

the morphological characters of head, thorax and abdomen. The analysis is in built into the computer menus, and at the end, larvae is correctly identified at the species level. This Technique has a potential to become an important training and in medical entomology.

PS - 5.4

A NEW SPECIES OF THE GENUS *DAVAINEA* BLANCHARD, 1891 (CESTODA : DAVAINEIDAE) FROM *FRANCOLINUS PONICE RIANUS.*

S R Patil[1], B V Jadhav[2] and G B Shinde[3]
1. Department of Zoology, Y C Warana Mahavidyalaya, Warananagar, 416 113, M S., India
2. Department of Zoology, Dr. B.A. Marathwade University, Aurangabad, 431 004, M S., India

The genus *Davainea* was established by Blanchard, 1891 from *Gallus domesticus*. Present paper deals with the description of a new species *D. farancolinusi* n.sp.in having the distinct characters such as scolex medium, oval, 0.121 to 0.364 in diameter ; rostellar hooks 16, single pronged, in a single circle; mature segments broader than long; testes 41 in number, oval, in a single field, more in preovarian field, a few on dorsal surface of ovarian lobes; cirrus pouch medium, obliquely placed, at 1/5th to 1/6th from anterior margin of the segments, ovary big, distinctly bilobed, mostly in the posterior half of the segments ; genital pores small, oval, unilateral, at 1/5th to 1/6th from the anterior margin of the segments.

PS - 5.5

R E Hayward
(Abstract not received)

PS - 5.6

BIODIVERSITY IN CERTAIN ROUND WORM OF SHEEP, RATS AND TAPE WORM OF BIRDS

Tikam Singh, Pradeep Kumar Sharma, Dusyant and S S Lal
Department of Zoology, C.C.S. University, Meerut - 250 005.

In case of both microparasites and macroparasites maximum cases of biodiversity are found (Cox, 1996). In the present study three new nematodes and one new cestode parasites have been described.
Bosicola coxii : Female - Females measured 18.88 - 18.93 (18.88) mm in length. The cephalic region contained 10 pairs of internal leaf crowns and 20 external leaf crowns. Male : Males measured 12.86 - 12.91 (12.87) mm in total length. Body cuticle, lateral lines and cephalic structures were similar to those of females. The spicules measured

1.3 - 1.6 (1.43) mm in length and 0.03 - 0.07 (0.047) mm in breadth. Copulatory bursa measured 0.28 - 0.32 (0.296) mm and 0.44 - 0.49 (0.47) mm respectively.

Bunostamum striatum : Female worms were 16 - 15.5 (15.75) mm and total breadth 0.58 - 0.56 (0.57) mm. Buccal capsule measured 0.14 - 0.15 (0.145) mm in length and 0.19 - 0.20 (0.195) mm in breadth. Length and breadth of oesophagus were 1.2 - 1.30 (1.25) mm and 0.34 - 0.30 (0.32) mm respectively. Body wall of these parasites was very peculiar in middle region forming an abnormal fan shaped or U-shaped external appearance. The epidermis along with the longitudinal musculature was found inverted U-shaped structure. Both cuticle and epidermis along with longitudinal muscle layers formed striated type of structure.

Heterakis smythii : This nematode was recovered from *Rattus rattus*.
Female : Measured 7.8284 - 15. 1384 mm. It was dorsally curved in anterior region. Total length of oesophagus was 0.8474 - 1.319 mm. Vulva was situated slightly below the oesophagus and measured 6.1527 - 6.9428 mm from posterior extremity.
Male : Males measured 6.68 - 9.74 mm in the anterior region. Mouth was surrounded by 3 lips. Present new nematodes contained pre-cloacal sucker and a very distinct post-cloacal sucker measuring 0.020 0 0.015 mm and 10 pairs of papillae. The spicules were equal in size and measured 0.322 - 0.4294 mm in length and 0.0210 - 0.0238 mm in breadth.

Raillietina cameronii : Reported from the WLH chicks of Meerut region. The tape worm measured 20-25 (22.5) cm maximum in length and breadth 0.818 - 1.32 (1.069) mm. Rostellum of parasite contained 42 hooklets in double row with irregular disposition. Gravid segments showed polymorphism.

PS - 5.7

QUANTITATIVE DISTRIBUTION OF NEMATODES ON PASTURE OF LITAN AND COMPARISON BETWEEN THE NEMATODES FAUNA OF TWO CLIMATICALLY DIFFERENT AREAS

Ch Dhanachand and N Mohilal
Parasitology Laboratory, Life Sciences Department, Manipur University, Canchipur, Manipur.

Quantitative distribution of three groups of nematodes - Tylenchs, Dorylaims and Mononchs are recorded in three different pastures i.e. unutilized pasture, utilized pasture and cattle fold. The result reveals that the Tylenchs have almost similar number in both unutilized and utilized pastures while in cattle fold their number decreases. Dorylaims have the highest population in cattle fold while the predatory nematodes are more in unutilized pastures.

A survey to determine the percentage frequency of occurrence for both phytophagous

and soil nematodes around the roots of *Cynodon dactylon* pers of Ukhrul and Chandeel Districts of Manipur revealed *Helicotylenchus, Aglenchus, Axonchium, Iotonchus, Parahadronchus* as a few among the most populated and *Boleodorus, Psilenchus, Pratylenchus, Hirschmanniella, Discocriconemella, Oriverutus* as among the least populated ones. The population of *Pratylenchus, Macroposthonia, Discocriconemella* are more in Chandel district while *Tylenchorhynchus, Axonchium* and *Oriverutus* are more in Ukhrul district. The lowest index of similarity is in between Chakpikarong and Moreh while the highest index of similarity is in between Ukhrul and Litan.

PS - 5.8

MORPHOLOGICAL FEATURES OF DIFFERENT NYMPHAL INSTARS OF POULTRY SHAFT LOUSE, *MENOPON GALLINAE* LINNE. (PHTHIRAPTERA : AMBLYCERA)

A K Sexena[1], S K Singh[1], Surman[1] and A. Kumar[2]
1. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Kotdwara (Pauri Garhwal), INDIA.
2. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Rishikesh (Dehradun), INDIA.

Poultry shaft louse, *Menopon gallinae* is a dreaded phthirapteran species infesting poultry. They do not only cause reduction in vitality and productivity of host bird but also act as transmitter and reservoirs of infectious diseases. Complete *in vitro* biology of these amblyceran phthirapterans has yet not been worked out. However, partial success has been obtained in rearing the nymphal stages of the louse, till the next moult. In present paper, an attempt has been made to provide comparative account of morphological features of the three nymphal instars and both the sexes of adults. In addition to body measurements, the nature of spiracles, brush/comb on their abdominal segments and legs, gular plate, hypopharynx and the appearance of genitalia have been taken into consideration. Such an account will help the phthirapteran biologists in identifying life history stages of above said louse.

PS - 5.9

SENGA MOHEKARAE N. SP. (CESTODA : PTYCHOBOTHRIDAE) FROM *MASTASEMBELLUS ARMATUS*

M B Tat[1] and B V Jadhav[2]
1. Department of Zoology, Arts, Commerce and Science College, Kille-Dharur.
2. Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431 004. Maharashtra, India

The present paper deals with the new species of *Senga mohekarae* n. sp. It differs from all the known species of the genus with characters like scolex, pear shaped, neck short,

number of hooks 22-26, the mature segments broader than long, number of testes 60-70, vagina starts from the common genital pore and vitellaria follicular.

PS - 5.10

A NEW CESTODE OF GENUS *DAVAINEA* BLANCHARD 1891, FROM *GALLUS DOMESTICUS* OF SOLAPUR, MAHARASHTRA INDIA

S V Kharade[1] and G B Shinde[2]
1. Department of Zoology, Krishna Mahavidyalaya, Shivnagar, Tal. Karad, Dist. Satara - 415 108.
2. Department of Zoology, Dr. B.A. Marathwada University, Aurangabad - 431 004, Maharashtra, INDIA

The study deals with description of new species *Davainea singhi.* The genus *Davainea* was erected by Blanchard 1891 (Davaine, 1860) with its type species *D. proglottina* from *Gallus domesticus. Davainea singhi* n. sp. characterised by scolex large, squarish, indistinctly marked off from strobila, with four suckers, oval, arranged in two pairs, in four corners of scolex, without spines ; rostellum large, oval, armed with 280-300 short hooks, in single circle. Neck is absent. Mature segments, broader than long with convex, unequal margins, with projections at posterior corners of segments. Testes medium, oval, 20-25 in number, post ovarian, unevenly distributed, in two lateral fields, in central medulla, bounded laterally by longitudinal excretory canals; cirrus pouch large, oval, obliquely placed, situated in anterior 1/4 of segments, directed anteriorly; cirrus wide, slightly coiled, protruded condition in few segments; vas deferens thin, medium length, slightly coiled, curved; ovary large, distinctly bilobed obliquely placed; ovarian lobes large, oval, each with 15-18, short, blunt, round acini; vagina, thin posterior to cirrus pouch, starts from genital pores, slightly swollen proximally, extends and opens in ootype; ootype small, oval, ventral to isthmus; genital pores, oval, irregularly alternate. Vitelline gland medium, compact, postovarian, slightly obliquely placed, transversely elongated.

PS - 5.11

ON A NEW SPECIES OF THE GENUS *PHYLLODISTOMUM* BRAUN, 1899 DIGENEA : GORGODERIDAE FROM THE STOMACH OF A FRESH WATER FISH *OMPOK BIMACULATUS* (BLOCH) AT VERANASI

Rezwan Ahmad, Veena Singh and A K Maurya
Ecology & Parasitology Laboratory, Department of Zoology, Banaras Hindu University, Varanasi - 221 005, INDIA.

Phyllodistomum bimaculatusi sp. nov. was obtained from the stomach of a fresh water fish *Ompok bimaculatus* (Bloch.) at Varanasi. The present form differs from all the other reported species of the genus *Phyllodistomum* Braun, 1899 from Indian fresh water fishes and comes close to *P. singhiai* in having mouth terminal. ventral sucker

larger than oral sucker, testes branched and lobed ovary. But it differs from it in having spatulate shape and smaller size of body and body organs, finely lobed ovary anterior to testes, testes nearly symmetrical, vesicula seminalis bilobed, uterus extending over caeca and non-operculated egg.

PS - 5.12

A NEW TAPEWORM FROM *WALLAGO ATTU* FROM A RIVER *GANESIA DHARURENSIS* NEW SPECIES

B V Jadhav[1] and M B Tat[2]

1. Department of Zoology, Dr. Babasaheb Ambedkar Maraathwade University, Aurangabad, 431004, Maharashtra., India

2. Department of Zoology, Arts, Commerce and Science College, Kille Dharur.

The present paper deals with the new species of *Gangesia dharurensis* n.sp. It differs from all the known species of the genus with the characters like, scolex globular, neck long, number of hooks 35-40, the mature segments broader than long, number of testes 60-70, vagina thin tube, ovary bilobed, long, finger like, and vitellaria granular.

PS - 5.13

ON A NEW SPECIES OF THE GENUS *LYTOCESTOIDES* BAYLIS 1928 FROM A FRESHWATER FISH

M G Babare[1] and B V Jadhav[2]

1. Department of Zoology, Arts, Science and Commerce College, Naldurg, Dist. Osmanabad., Maharashtra, India

2. Department of Zoology, Dr. B.A. Marathwada University Aurangabad 431 004, Maharashtra, India

The present paper deals with a new species of *Lytocestoides follicularis* n.sp. It differs from all the known species of the genus with the characters like shown head without longitudinal furrows, testes 45-50 in number, preovarian, oval, in the anterior half of the worm, anterior to cirrus pouch, medullary placed, ovary bilobed with equal lobes, uterus with numerous eggs and cirrus pouch oval.

PS - 6.1

MALARIA AMONG MIGRANT LABOURERS : A SOCIO - EPIDEMIOLOGICAL INQUIRY

Madhukar Pai, Anand Zachariah, Winsley Rose, Samuel Satyajit, Santosh Verghese and Abraham Joseph
Department of Community Health and Medicine, Christian Medical College, Vellore, India

Migrant malaria is now considered an epidemiologically distinct type of malaria. Recent epidemics have been linked with population movements and man-made ecological changes. This is a study of a group of migrant labourers who were screened after coming into Vellore town from a malarious area. Altogether 41 out of the group of 48 had confirmed malaria (falciparum accounted for more than half). Many were acutely sick and severely anaemic. Because they were dislocated and powerless, they were denied timely, adequate health care. From a public health view point, the group was a large falciparum reservoir in an area where falciparum was not endemic. Since they had come into Vellore from an area suspected to have chloroquine resistant *P. falciparum,* there was concern about the spread of drug resistant malaria in a vulnerable population. While this concern was justified, the negative reaction of the local community probably led to the eviction of the migrant group from the town even before the completion of their treatment. The study pointed to the possibility of imported cases being a major source of infection for Vellore where the malaria incidence had reached alarming proportions. On the other hand, the study raised social and ethical issues in health care of migrants and highlighted the need to consider migrant as vulnerable, high risk people with unique problems. This study reflects the importance of widening socio - economic disparity, unchecked development and ecological devastation in the resurgence of malaria in India. There is an urgent need to consider these social realities while planning for malaria control in India. The need for clear policy on migrants and their health care is also discussed.

PS - 6.2

ASSESSMENT OF ECONOMIC LOSS DUE TO MALARIA IN RAIGAD DISTRICT, MAHARASHTRA

V S Malaviya, P G Bhati and R S Yadav
Malaria Research Centre (ICMR), Field Station, Civil Hospital, Nadiad-387 001, Gujarat, India

Raigad district in coastal Maharashtra with a population of 1.5 million, is a relatively less developed area and is mainly inhabited by backward classes and scheduled castes. As part of study on malaria during 1996, we made an assessment of the economic loss due to malaria which is endemic in the region. Annual expenditure on health per family was estimated at Rs. 526 (US $ 14.4). Based on a questionnaire survey among 132

persons who had suffered from malaria in the recent past, which included the costs on treatment, travel and loss of wages of patients and attendants, mean total loss per episode of malaria was estimated at Rs. 315 (US $ 8.8). There were variations in economic loss in different PHCs, the reasons of which will be discussed. Although the amount of human sufferings is beyond any monetary estimation, the total economic loss due to malaria in the district was estimated at Rs. 4.9 million during 1995 which is very significant. Community expenditure on health/ malaria will be discussed.

PS - 7.1

EFFECTIVE MANAGEMENT OF DIARRHOEA CAUSED BY PROTOZOON AND ENTEREOPATHOGENS IN LABORATORY NON-HUMAN PRIMATES

D C Purohit, S.N.A. Rizvi and D.S. Rathore
National Laboratory Animal Centre, Central Drug Research Institute, Lucknow.

Diarrhoea is the most common clinical manifestation in laboratory non human primates and may result in a considerable morbidity and mortality. Survey conducted during the last three years at National Laboratroy Animal Centre, C.D.R.I., Lucknow revealed *Entamoeba histolytica, Giardia, Escherichia coli* and *Enterobacter* as the most common causative agents involved in diarrhoea. In the present study animals suffering from diarrhoea were placed in three groups. Diarrhoea caused by protozoon in Group A, enteropathogens in Group B and mixed infections in Group C. Group A was treated with Metronidazole, Group B with Trimethoprim-Sulphamethoxazole and Group C with combination of Metronidazole and Ciprofloxacin. The antimicrobial agents were administered orally by Rayle's tube for one week. Recovery rate observed was : 72% with Metronidazole. 89% with Trimethoprim-Sulphamethoxazole and 96% with combination of Metronidazole and Ciprofloxacin. This study indicates that the combination of Metronidazole and Ciprofloxacin can be considered as the antimicrobial agent of choice for diarrhoea in laboratory non-human primates, particularly in mixed infections.

PS - 7.2

REDUCTION OF LARVAL DENSITY OF *TRICHINELLA SPIRALIS* IN MUSCLES OF MICE BY *CINA, CALCAREA FLUOR* AND *THUJA*

Rintu Sarkar, Santi P Sinha Babu and Nirmal C Sukul
Department of Zoology, Visva-Bharaati University Santiniketan-731235, West Bengal, India

Swiss albino mice were first treated with hydrocortisone for 15 days and then inoculated with *T. spiralis* larvae. Thirty days after inoculation one group of mice was treated with a high-dilution of *Artemisia absinthium* extract (*Cina* 1 M). The second group with *Cina* 1M followed by a high dilution of *Thuja occidentalis* (Thuja CM). The third group served as untreated control. Mice treated with dilutions of *Cina* and *Thuja* showed significant reduction of larval population in their muscle. *Cina* alone showed some reduction in larval population but the effect was smaller as compared with the combination treatment.

PS - 7.4

ANTIFILARIAL EFFECT OF A COMBINATION OF BOTANICALS FROM *ACACIA AURICULIFORMIS* AND *CENTELLA ASIATICA* ON CANINE DIROFILARIASIS

Paromita Sarkar, Santi P. Sinha Babu and Nirmal C. Sukul
Department of Zoology, Visva-Bharati University, Santiniketan -731 235, West Bengal, India

A mixture (1:1) of the ethanol extracts obtained from the leaves of *Centella asiatica* and from the funicles of *Acacia auriculiformis* was administered orally on pariah dogs naturally infected with *Dirofilaria immitis* at 0.04 mg/g/d for 45 days. Two triterpenoid saponins, acaciaside A and B, isolated from the funicles of *A. auriculiformis* were combined with the ethanol extract from the leaves of *C. astiatica* (1:1) and tested at 0.036mg/g/d for 45 days on microfilaraemic dogs. There was an initial fluctuation in the mf count in the blood following treatment with the mixture of crude extracts and then the mf density decreased upto 99%. The suppression lasted for 120 days. When treated with the saponins mixed with the ethanol extract from *C. asiatica,* there was 86% reduction in mf density 30 days after the last date of treatment and 68% reduction was maintained even 120 days after the treatment.

PS - 7.5

EFFECT OF SAPONINS FROM *ACACIA AURICULIFORMIS* ON CANINE DIROFILARIASIS AND MURINE TRICHINOSIS

Anirban Sukul, Rintu Sarkar and Santi P Sinha Babu
Department of Zoology, Visva-Bharati University, Santiniketan-731 235, West Bengal, India

Triterpenoid saponins isolated from the funicles of *A. auriculiformis* were administered orally on pariah dogs infected with *Dirofilaria immitis* at 15mg/Kg body weight / day for 30 days. Microfilarial concentration was significantly reduced from day 30 counted from the onset of treatment onwards. The saponins were also administered orally at 90 mg/kg/day on albino mice inoculated with *Trichinella spiralis* on days 4, 5 and 6 from the date of inoculation. There was a significant reduction in adult population of *T. spiralis* in the intestine of mice examined on day 10 from the date of inoculation. Controls in both cases did not show any significant change in their respective parasite load.

PS - 7.6

ANTIFILARIAL EFFECT OF A PLANT *CARICA PAPAYA* L.

Naba K Ghosh, Santi P Sinha Babu, Nirmal C Sukul
Department of Zoology, Visva-Bharati University, Santiniketan - 731 235, West Bengal, India

Air-dried and powdered unripe fruits of *Carica papaya* was administered orally at 60 mg/kg/day for 60 days on two pariah dogs naturally infected with *Dirofilaria immitis*. Microfilarial density in the blood rose gradually following the onset of treatment, a 12.3 fold increase being recorded on day 30. Thereafter, the count was reduced by more than 81 on day 210. It did not produce any appreciable side effects in the treated dogs. The maintenance of the reduced level of microfilarial density may be due to the sizeable reduction of adult worm loads. The effect of *C. papaya* on *D. immitis* of dogs has been discussed at length.

PS - 7.7

CESTOCIDAL ACTIVITY OF *GLADIOLUS SP.*

Arpita Saha, Naba K Ghosh, Santi P Sinha Babu and Nirmal C Sukul
Department of Zoology, Visva-Bharati University, Santiniketan-731 235, West Bengal, India

Ethanol extract obtained from the bulbs of *Gladiolus sp.* was administered orally to a batch of 10 albino rats infected with *Hymenolepis diminuta* at 300 mg/kg/day for 15 days. The rats were starved for 24 hrs before treatment. All the rats were infected with a single cysticercoid of *H. diminuta*. The average number of discharging eggs decreased significantly after treatment. The eggs disappeared totally from the faeces of rats 30 days after treatment. No adult worms were recovered from the treated rats on autopsy. Adult worms recovered from the control rats were found to be actively motile. No apparent toxic effects were observed in the rats. It is evident from the results that the plant possesses strong cestocidal activity against *H. diminuta* in rats.

PS - 7.8

IN VITRO CULTURE SYSTEM AS A TOOL FOR SCREENING XANTHINE ANALOGS AS POTENTIAL ANTIMALARIAL

Vidya Prasanna Kumar, Santanu Datta, Suresh M. Solapure.
Astra Biochemicals (P) Ltd., Malleswaram, Bangalore, India.

As *Plasmodium falciparum*, the cause of most of the severe cases of malaria, is increasingly resistant to available drugs, there is an urgent need to identify new targets for chemotherapy. HGPRT, an enzyme involved in the salvage pathway of the parasite, is one of the potential targets. The inhibition of Pf HGPRT will block the conversion of

hypoxanthine to inosine monophosphate. We have screened various HGPRT inhibitors on *P. falciparum in vitro* culture system where the growth of the parasite is monitored by [^{3}H] hypoxanthine uptake. Since inhibition of Pf HGPRT will affect the incorporation of [^{3}H] hypoxanthine, it might give a false indication of growth inhibition. To overcome this effect we have used [^{3}H] ethanolamine as an alternative growth marker. Results indicate that several inhibitors do have identical IC_{50} value proving the essentiality of HGPRT to the parasite. In the screens we have also found some inhibitors that show stage specific inhibition. In order to identify the growth stage at which Pf HGPRT is expressed, total RNA from synchronous culture at different stages was isolated and followed by northern blot analysis.

PS - 7.9

ANTHELMINTIC EFFECT OF JUGLONE ON MATURE AND IMMATURE *HYMENOLEPIS NANA* IN MICE

L B Dama and B V Jadhav
Department of Zoology, Dr Babasaheb Ambedkar Marathwada University, Aurangabad 431 004, M.S., India.

The ability of treatment schedules of herbal 'Juglone' (5-Hydroxy-1, 4, - Napthoquinone) to kill well established immature and mature *Hymenolepis nana* was studied. The mice infected with *Hymenolepis nana,* were treated with Juglone at a dose rate of 50 µg/kg/ day body weight. Immature worms are affected by Juglone administered for 12 consecutive days (days one to 12 after infection). The drug Juglone given at 50 µg/kg/ day fro five consecutive days after (days 12 to 16 after infection) eliminated 100% of mature worms. Juglone was significantly effective against *Hymenolepis nana.*

PS - 7.10

STREBLOFIL : EFFECT ON MACROFILARIIDS

Arvind K Srivastava, R K Chatterjee, D K Kulshreshtha and B N Dhawan
Central Drug Research Institute, Lucknow 226 001

Streblofil, the standardized glycosidic fraction from the bark of *Streblus asper*, is macrofilaricidal and has a novel biochemical mode of action. Streblofil causes death or sterillization of the *Acanthocheilonema viteae* or *Setaria Cervi in vivo* and their death *in vitro*. Streblofil produces a severe decline in intracellular reduced glutathione pools, glutathione-s-transferase and glutathione peroxidase activities in the filariids. This leads to reduction in microfilarial production, motility and ability to protect themselves against attack by reactive oxygen species including the secondary products of lipid peroxidation. The other major effect of streblofil is impairment of energy producing system of the parasite. Streblofil inhibits the glucose uptake and glucokinase activity of the filariids which may be resulting in reduced motility and paralysis. Streblofil is unique in that it is not dependent on host immune system for its macrofilaricidal activity.

PS - 7.11

SYNTHESIS AND *IN-VIVO* ANTIMALARIAL ACTIVITY OF 6-CYCLOPENTENYL-1, 2,, 4-TRIOXANE.

S Chandra[1], G Saxena[2], C Singh[2] & J C Katiyar
1. Division of Parasitology, C.D.R.I., Lucknow.
2. Division of Medicinal Chemistry, C.D.R.I., Lucknow.

Introductiono f Artemisinin, a sesquiterpene peroxide dervied fro *Artemisia annua* is highly potent antimalarial agent and active against both chloroquine sensitive and CQ-resistant malaria. Several derivatives of artemisinin are currently being developed as drug. The presence of peroxide group in the form of 1, 2, 4 - trioxane is essential for natimalrial activity of these compounds.

A series of new 6-cyclopentenyl-1, 2, 4-trioxiane has been synthesized and evaluated for antimalarial action agaisnt *Plasmodium berghei* in mice. Male Swiss mice of 18±2 g body weight were inoculated with $1x10^6$ parasitized red blood cells. Two hours after the infectiion, test compounds were given i.p. at 90, 30 mg/kg x 4 days, twice daily for four consecutive days. Parasitaemia was recorded after twenty four hours of last drug administration then on day 7, 9, 11, 15, 20 and 28. Amongst the seven compounds tested, parasitaemia till 28th day at 90 mg/kg. *In vivo* antimalarial activity of 6-cyclopentenyl 1,2,4-trioxanes a new class of 1,2,4-trioxianes will be presented.

PS - 7.12

B V Dharmendra
(Abstract not received)

PS - 7.13

M Singh
(Abstract not received)

PS - 7.14

MANAGEMENT OF GIARDIASIS BY HERBAL DRUG : *CENTELLA ASCIATCA* LINN

Shashi Prabha, D M Tripathi , Pramid Nath, A K Agrawal
Post Graduate Department of Pathology , K.G. Medical College , Lucknow

The incidence of Giardiasis is alarmingly high, especially in the regions of world with inadequate hygienic conditions especially in population of lower socio-economic levels. Large proportion of patients remain asymptomatic. The modern drugs,though posses quick response on acute infection, however, these are associated with severe side

effects . Herbal drugs, on the contrsry carry promising scope to control these parasitic infections. The present study deals with a herbal drug, Mandookparni (*Centella asciatica*) , a seasonal herb found at moist and shaddy places in tropical regions.
Herbal power, was prepared by grinding the whole plant after its collection , thorough washing and drying in shade. *Herbal syrup,* prepared by grinding the fresh plant, collecting the juice , cooking for 30 min. with sugar, dispensing in bottles and sealing with wax. Syrup, so prepared, was dispensed in patients suffering from giardiasis, 2-3 ml per dose TDS for 3-7 days in children of 1-5 years of age . Caapsules, filled with whole plants of 5-15 years and 2 capsules / dose TDS for those above 15 years of age for 3-7 days.

Demonstrable reeovery from infection was observed in >82% patients after 3 days treatnent, either with the syrup or capsules prepared from *centella asciatica.* 12.5% patients recovered after 7 days treatment. 5% patient did not respond to the drug.

Inferences are drawn from the study that *Centella asciatica,* as a herbal drug offers a phenomenal recovery fron Giardiasis due to *Giardia lamblia,* since no side effects are recorded. Hence this herbal preparation appears to be a drug of choice over the conventional modern drugs. Mode of action of *Centella asciatica* is under investigation.

PS - 7.15

DRUG RESISTANT MALARIA COMPLICATING LEUKAEMIAS AND LYMPHOMAS IN CHILDREN

Nirmala, Archana Kumar[1], A K Kapoor, B Lal, G P Dutta[2] and P K Misra[1]
Department jof Pathology & Paediatrics, KG's medical College, Lucknow.
Division of Microbiology, CDRI, Lucknow.

Clinical studies on malaria in children have shown high frequency of drug-resistant malaria associated with malignanvies such as leukaemia and lymphomas. Amongst the malignancy group of 50 cases, 62% (31 of 50) were diagnosed as to be having *P. falciparum* infection. There was a high preponderance of drug-resistance against chloroquine (51.62%) among malaria with malignancy group as compared to 23.7% (9 of 38) in control (malaria without malignancy) group. In addition, resistance was also encountered against metakelfin (pyrimethamine + sulphamethopyrazine) in 46.1% in malaria with malignancy group as compared to 28.5% in the malaria without malignancy group. No resistance to quinine was encountered during the entire period (1994-1995) of this study.

PS - 8.1

INVESTIGATION ON MALARIA IN A TRIBAL AREA OF VALSAD DISTRICT, GUJARAT

H C Srivastava and R S Yadav
Malaria Research Centre (ICMR), Field Station, Civil Hospital, Nadiad-387 001, Gujarat, India

Malaria continues as an important public health problem in Gujarat state in western India. From 1989 there has been a gradual decline in malaria incidence in the state. During 1995 there were some deaths in Sutharpada PHC in Valsad district in southern Gujarat. Sutharpada had forested hills which are mainly inhabited by aboriginal tribals (>54%). An investigation in malaria was carried out in this area in November 1995. *An. culicifacies* and *An. fluviatilis* were found resting in the houses and breeding in hill streams and river courses. Retrospective analysis of malaria from PHC records showed that malaria API nearly doubled from the lowest of 3.7/1000 in 1994 to 6.8/1000 in 1995. In the villages where deaths occurred, malaria parasite rate was found to be 26%; *P. falciparum* accounted for 80% infections and the rest were *P. vivax*. It was found that compared with a minimum norm of 10% blood slide collection from the population, there was a total breakdown of active surveillance which led to a build up of parasite load in the community. There was a focal outbreak of *P. falciparum* which led to the death. In difficult terrain there is a need to strengthen case detection mechanism, laboratory services and IEC and provide easy access to antimalarials to reduce morbidity and avert deaths. In view of the widespread resistance in *An. culicifacies* to DDT, a change of insecticide strategy in favour of effective insecticides is warranted.

PS - 8.2

GENETIC HETEROGENEITY WITHIN *ECHINOCOCCUS GRANULOSUS* ISOLATES CHARACTERISED BY RANDOM AMPLIFIED POLYMORPHIC DNA-POLYMERASE CHAIN REACTION

Y A Reddy, J R Rao, S K Bandyopadhayay, Bhaskar Sharma and G Butchaiah.
Faculty of Biotechnology, Indian Veterinary Research Institute, Izatnagar - 243 122, India

To determine the distinctiveness of *Echinococcus granulosus* of dog-cattle and dog-buffalo cycle, DNA polymorphisms awere assessed by amplifying the genomic DNA with single nonspecific primers. The genomic DNAs were amplified by the use of 10 to 11 base arbitrary sequence primers with a GC content of 60 to 81.6% to generate polymorphic DNA. Amplification reactions were performed in 50µl volumes containing 5µl reaction buffer, 200µM each of dNTPs, 15p moles of primer, 10 ng genomic DNA and 1.8 units of Taq DNA polymerase. The DNA was initially denatured for 4 min at 94°C and the PCR cycles was carried out for 1 min at 94°C, 45 Sec at 36°C and for 1 min at 72°C for a total of 40 cycles followed by one extensive polymerisation reaction (5 min

at 72°C). A typical RAPD amplification yielded 9-16 DNA fragment ranging from 260 to 2200 bp on 1.4% agarose gels. The two strains of *E. granulosus* showed minor and major difference in their amplified DNA patterns notably with AP2 primer (5' - TGCCGAGCTG-3). A DNA fragment of approximately 900 bp reamplified from the genome of *E. granulosus* of Buffalo organ was DIG labelled and used to examine the strain differentiations. Dot-blot hybridization of total genomic DNA differentiated *E. granulosus* isolates from cattle and buffaloes. No detectable cross-hybridization signal was seen with heterologous cattle *E. granulosus* DNA and the host genomic DNA of buffalo origin.

PS - 8.3

ANAEMIA ASSOCIATED WITH BUBALIAN FASCIOSLIOSIS

R L Sharma[1], S C Yadav, Kalicharan[2], U R Mehra[3] R S Dass[3] and A K Varma[3]
1. Division of Parasitology Indian Veterinary Research Institute Izatnagar - 243 122, India.
2. Division of Pathology, IVRI, Izatnagar.
3. Division of Animal Nutrition, IVRI, Izatnagar.

Investigations on anaemia caused by *Fasciola gigantica* in experimentally infected buffalo male calves with 1000 metacercariae aged 6-8 months, of Murrah breed, were made. Haematological parameters measured weekly over a period twenty four weeks post infection (PI), showed a significant fall in erythrocyte counts and haemoglobulin levels, after the flukes entered into bile ducts (9th week PI) and a significant increase in total leucocyte counts in infected group of calves than control. The alterations started from 3rd week PI and continued to remain different till the end of experiment (24 week PI). However in uninfected controls the values of the above parameters though oscillated until the end of study but were observed within the normal range for male buffalo calves. The developing flukes caused 70% mortality in the infected group. Various factors contributing to high mortality due to *F. gigantica* were (a) traumatic injury to hepatic parenchyma, (b) blockage of portal circulation, (c) excessive interference with hepatic functions by the parasite and (d) anaemia induced generalised oxygen starvation at tissue level in the infected host. Thus, the aneamia associated with bubalian fascioliosis needs further confirmation as to whether it is caused by haematophagous activity of the parasite, effect of toxic substances emanating from flukes, generalised oxygen starvation at tissue level or a synchronous/amalgamated effect of all these factors.

PS - 8.4

HOOKWORM INFECTION : A CROSS-SECTIONAL STUDY OF A RURAL COMMUNITY IN A SUBTROPICAL, HIGH-RAINFALL AREA OF MEGHALAYA, INDIA

L M Lyndem, V Tandon and A K Yadav
Department of Zoology, North-Eastern Hill University, Shillong - 793 022, India.

An intensive survey for hookworm infection was carried out in a village in Meghalaya state (North-East India). The study population was a village community (Nongkya, Umsning), comprising 489 individuals. Only 425 subjects could be examined as the others declined to participate in the study. Stool examinations were made using Kato-Katz smear technique and modified Harada-Mori test-tube cultivation methods. Of the tested subjects, 58.5% harboured single-or multiple-species infections involving hookworm, *Ascaris* and *Trichuris*. The prevalence of hookworm infection occurring as a single-species infection was 7.29%. The study identified the high-risk age group for this infection as 5-9 years, since the prevalence was higher in this age group (13.9%) in comparison to the age groups 0-4 years (3.48%), 10-14 years (10.4%), 15-19 years (9.52%) and 20+ years (3%). Between the two sexes, the prevalence was higher among females. The hookworm infection was found largely restricted to the subjects who worked in the field. The influence of ambient climatic factors on the hatching of eggs and viability of infected larvae is being assessed.

PS - 8.5

SEASONAL PREVALENCE OF ANOPHELINES IN SHAHJAHANPUR DISTRICT, UTTAR PRADESH.

S N Sharma and R P Shukla
Malaria Research Centre, Field Station, Inderjeet Garden, Bhotia Parao, Haldwani- 263 141 District Nainital.

Information on anopheline prevalence particularly the vector species is required for proper planning of malaria control activities. Practically very little has been published regarding the seasonal prevalence of different anophelines in Shahjahanpur district where malaria epidemics had occurred in the past. Longitudinal surveys from Feb. 1987 to Dec. 1991 were carried out for collection of adult mosquitoes from 2 villages of Shahjahanpur. Results revealed prevalence of 10 anophelines in the area. Specieswise percentage composition was. *An. annularis* 39.2%. *A n. subpictus* 31.5%, *An. aconitus* 15.0% *An. culicifacies* 12.9%, *An. pallidus* 0.5%, *An. splendidus* 0.5%, *An. barbirostris* 0.2% , *An. nigerrimus* 0.2%, *An. stephensi* 0.1% and *An. vagus* 0.02%, An. anophelines, *viz; An. culicifacies, An. annularis, An. subpictus* and *An. aconitus* were predominantly prevalent. The other above mentioned 6 species were found in low number. Both *An. culicifacies* and *An. annularis* prevailed all through the year with densities ranging from 0.8 to 37.2 and 1.0 to 106.0 respectively. High density of *An. culicifacies* was found during July to Sept. and low during Jan and Feb., *An. annularis* prevailed with higher densities during Oct. to April. The prevalence pattern of *An. aconitus* was similar to that of *An. annularis.*

High densities of *An. subpictus* occurred during July to Sept. and low during Jan. to April. *An. splendidus, An. pallidus, An. nigerrimus* and *An. barbirostris* prevailed during post-monsoon and winter months. Both *An. stephensi* and *An. vagus* showed erratic

prevalence. Overall results of the 5 year study revealed marked difference in the seasonal prevalence of different species.

PS - 8.6

LYMPHATIC FILARIASIS IN TWO DISTINCT COMMUNITIES OF UPPER ASSAM

A M Khan, P Dutta, S A Khan, P K Mohapatra, N K Baruah, C K Sharma and J Mahanta
Division of Entomology and Filariasis, Regional Medical Research Centre, NE Region (ICMR), Post Box No. 105 Dibrugarh-786 001 Assam, India.

Prevalence study on lymphatic filariasis in two different communities (tea labourer and non-tea labourer) having distinct social and racial background but living in the same areas and experiencing similar mosquitogenic environment in an endemic population of Assam was conducted. A total 1357 night blood slide samples from both the groups by random sampling (> 15% coverage) were collected and examined for microfilariae. Out of the total 779 sample from tea labourers, 80 were found positive (10.3% mf rate) with a mean mf density 36.6. Among non tea labourers, only 10 were found positive of the total 578 subjects screened (1.7% mf rate) with a mean mf density 28.9. Among the non-tea labour population interestingly majority of the cases were migrants and only three local Assamese were positive. Though active transmission in the study area was confirmed by isolating infective vector mosquito *Culex quinquefasciatus* intake of infection among migrants is not very clear as they frequently visit their native places which are also endemic for filariasis. Further, all 11 subjects with full clinical manifestation recorded, belong to tea labourers group only. Significant difference in mf carriers and clinical manifestations of the two groups suggest involvement of some social / behavioural / genetic factors. Details of these factors will be discussed.

PS - 8.7

IDENTIFICATION OF *ENTAMOEBA HISTOLYTICA* BY METHOD BASED ON DIRECT PCR AND RAPD ANALYSIS

Alka Prakash, A Chakroborti, R C Mahajan and N K Ganguly
Department of Exp. Med. and Biotech, Department of Parasitology, PGIMER. Chandigarh, India-160012.

Amoebiasis, caused by the protozoan parasite *Entamoeba histolytica* still remains a major cause of morbidity and mortality in the developing countries. The pathogenesis of this parasite results in severe ALA, intestinal amoebic colities, dysentery and asymptomatic carrier state, due to harbouring of pathogenic or non pathogenic strains of *Entamoeba*. In the present study, an attempt has been made to differentiate *E. histolytica* and *E. dispar* by using certain sequence specific oligonucleotide primers in PCR system. The parasite DNA directly from number of clinical sample (without prior cultur-

ing of parasite) has been used for DNA amplification with different oligonucleotide primer sets. Some important genes like cysteine Proteinase, interestingly showed increased copy number in cased of ALA whcih can be directly correlated with virulence of the strainin ALA pathogenesis as comared to the intestinal situation. The Ribosomal (SSU) rRNA gene also has certain distinct sequences which have prooven to be helpful a differentiating parasite strains with clinical samples in PCR based generation of distinct RELP pattern. Also, Random amplified polymorphic DNA. PCR by using Single 18S or 28S rRNA primer sequence generates different banding patterns with number of clinical samples. Such results indicate that by using even minute amount of DNA important parasite genes can be used in differentiation of strains directly at the molecular level which is needed in order to develop molecular diagnostic approaches.

PS - 8.8

MALARIA IN NORTH EASTERN REGION OF INDIA : AN OVERVIEW IN RETROSPECT

P K Mohapatra, Anil prakash, D R Bhattacharyya and J Mahanta
Regional Medical Research Centre, N.E. Region (ICMR), P.O. Box - 105 Dibrugarh - 786001, Assam

The paper analyses the malaria situation in N.E. States of India since 1961 to understand the pattern of changing epidemiology in space and time. The seven highly malarious North-eastern states, predominantly tribal, hilly with wet tropical forest share 3.7% of population of India but contribute up to 10% of total malaria burden, 17% of *P.falciparum* infection and a whopping 50% of deaths due to malaria in the present Indian scenerio. The worsening of malaria situation is evident from the fact that while population of the region has grown by 2.35 fold, the corresponding jump in quantum of malaria has been to the tune of 21 fold and that of *P.falciparum* over 51 times. The Pf proportion has increased from 32% to 60% during this period (1961-95). Though between 1981 and 1990 the malaria incidence registered a downward trend which however, was not reflected in Pf incidence. The situation of antimalarial drug resistance is also alarming. Various studies indicated the enormous increase of chloroquine resistance over a period of time from 37% (1978-82) to 56% (1988-93) in addition to confirmed resistance of parasite to sulphadoxin - pyrimethamine combination drugs and declining efficacy of quinine. The situation in various malaria paradigms existing in North-eastern region will be discussed in the light of changing ecological and vector bionomics.

PS - 8.9

J S Rao
(Abstract not received)

PS - 9.1

ROLE OF HUMAN POPULATION IN MALARIA TRANSMISSION

H Prasad[1] and D K Jain[2]
1. Malaria Research Centre, Field Station, Shahjahanpur, U.P.
2. Bareilly College, Bareilly, U.P. India.

To assess the role of human population in Malaria transmission, some important aspects viz parasitological, community response at the time of spraying houses with insecticides, housing and clothing pattern and movement of population were studied in district Shahjahanpur, Uttar Pradesh. The parasitological observations reflects that 14.5% malaria patients showed behavioural indifferences and ignorances towards the treatment of malaria. Such patients either took improper or delayed treatment which resulted in persistence of malaria in the community. Due to refractory response of people at the time of insecticidal spraying of houses, only 27.2% houses could be sprayed completely. However, the rest 61.2% and 11.4% houses remained partially and unspread respectively leading to higher vector density i.e. 69 and 43 per man hour in unsprayed and partially sprayed houses respectively which were sufficient enough to transmit malaria more as compared to completely sprayed houses where vector density was found 11 per man hour. The type of houses and its association with cattle sheds were found influencing malaria. The risk of aquairing malaria in thatched houses was found 16% as compared to 10.5% in pukka houses. It was further observed that the risk of malaria was found higher 13.8% in human dwellings as compared to 6.8% in human dwellings associated with cattle sheds. Clothing and covering of the body was also found playing an important role in malaria transmission. Males were found more exposed to mosquito bites as compared to females because of their dress like dhoti, trousers, kurta/shirts, baniyan, underwear/half pants, etc. and saree, blouse, salwar, kurta, etc. of the male and female respectively. In our study areas movement of population was very limited, therefore, it was not found playing any significant role in Malaria transmission.

PS - 9.2

LIPID PERCENTAGE VARIATION ACCORDING TO THE SEASONS IN *MONIEZIA* (B) *JADHAVAE* FROM *CAPRA HIRCUS* AT KALLAM, DISTT. OSMANABAD, MAHARASHTRA, INDIA

C J Hiware and A D Mohekar
P.G. Department of Zoology, Shikshan Maharshi Dayandeo Mohekar Mahavidyalaya, Kallam Distt. Osmanabad Maharashtra-413 507, India.

The present communication deals with the lipid content of *Moniezia* (B.) *Jadhavae* n.sp. during September, 1990 to March, 1991. The lipid percentage was obtained with different degrees showing the dependence of the parasite for its development on the nutrition of the host which varies according to season. The experimental result shows slight differences in the percentage of lipid during seven months.

PS - 9.3

ACCUMULATION OF HCH AND DDT RESIDUES IN SKIN LIPIDS

C S Pant,[1] V K Dua[2] and V P Sharma[3]
1. Malaria Research Centre, Civil Hospital, Nadiad-387 001;
2. Malaria Research Centre, BHEL, Hardwar.
3. Malaria Research Centre, 22 Sham Nath Marg, Delhi, India

Technical and ethical problems often prevent the collection of human blood, milk and adipose tissue samples under field conditions. Therefore, skin lipid was first time used in India as a bioindicator to monitor pesticide body burden in different population groups. Skin lipid samples collected from insecticide sprayed area of Mathura district (15 of occupationally exposed group and 24 of general population) and bioenvironmental control area of BHEL, Hardwar (29) were analysed for HCH and DDT residues by gas chromatographic technique. The mean residual concentrations of the total HCH and DDT concentrations in skin lipid samples of occupationally exposed people were 54.59 ± 20.50 and 27.17 ± 19.66 mg / kg, respectively compared to 17.92 ± 12.40 mg /kg of total HCH and 13.86 ± 12.70 mg/kg of total DDT in lipid samples from the general population. Similarly the mean residual concentrations of total HCH and DDT in unexposed (bioenvironmental control area) population were 5.07 ± 4.21 and 1.86 ± 1.76 mg/kg respectively. This results of total HCH and DDT residues showed a significant difference ($p < 0.05$) in the mean values between 1) occupationally exposed group and general population and 2) unexposed and general population. The correlation between lipid and blood was highly significant for HCH and DDT residues in the general population but was low to insignificant for occupationally exposed people suggesting that skin lipid analyses can provide a suitable bioindicator for monitoring HCH and DDT accumulation in the general population.

PS - 10.1

CHARACTERIZATION OF PROTEASE(S) IN ADULTS OF *TRICHURIS GLOBULOSA* (NEMATODA : TRICHURIDAE)

D K Kocher, S P Ahuja and M L Sood
Departments of Zoology and Biochemistry, Punjab Agricultural University, Ludhiana-141 004, India.

Activity of protease(s) has been observed separately in male and female *T. globulosa.* Different fractions showed optimum protease activity at 37°C, pH 9.5 and 1.5 mg casein concentration. E/S products showed maximum protease activity, which might be helpful in the degradation of mucosal tissue of the host gut. Male parasites appeared to feed extensively on connective tissue collagen / elastin, while females both on connective tissue and body / cellular fluids. Inhibition / activation studies revealed the presence of four kinds of protease(s) in the E / S products, cytosolic and membrane bound fractions performing different functions.

PS - 10.2

TRYPANOSOMA BRUCEI : PURIFICATION OF RELEASED 63 kDa PROTEASE

Daniel M N Okenu[1], *Kenneth N Opara*[2] and Mark Nwagwu[3]
1. Division of Biochemistry, National Institute for Medical Research, PMB 2013, Yaba, Lagos, Nigeria.
2. Dept. of Zoology, University of Uyo, PMB 1017, Uyo Nigeria.
3. Dept. of Zoology, University of Ibadan, Ibadan, Nigeria.

Thrombocytopaenia or platelet aggregation is a serious complication of African trypanosomiasis. The biochemical basis is not clearly known, but it is independent of complement, kinin, antibody, adenosine diphosphate or fatty acids. Proteases are known potent inducers of blood coagulation and platelet aggregation, and unknown factors released by *T. brucei* have been shown to induce platelet aggregation. Towards defining the biochemical mechanisms involved in thrombocytopaenia, we purified and characterised a major proteolytic enzyme released extracellularly by *T. brucei.* Actively motile trypanosomes released proteins into the medium (phosphate/saline/glucose, pH 8.0) in which the organism were incubated in *vitro*. The M_r of the released polypeptides ranged from 15 kDa to >200 kDa, amongst which are proteases. One of the major protein bands, a 250 kDa protease was purified to apparent homogeneity by ammonium sulfate precipitation, followed by DEAE-cellulose chromatography and Sephacryl S-300 gel filtration. The protease migrated as a single band of 63 kDa upon electrophoresis in both denaturing gel, and non-denaturing gel co-polymerised with gelatin. The enzyme was strongly active against Z-ARR-AFC peptide substrate, with a pH optimum of 7.0. The proteolytic activity was enhanced by dithiothreitol, and inhibited by E-64,

leupeptin, TPCK and antipain. The proteolytic enzyme is putativily identified as a cathepsin B-like cysteine protease.

PS - 10.3

STUDIES ON AMINO ACID TRANSPORT AND ABERRATIONS IN CEREBRAL MICROVESSELS DURING *PLASMODIUM YOELII* INFECTION IN MICE

Anju Agarwal[1], L M Tripathi[2], A K Kapoor[1] and V C Pandey
1. Division of Biochemistry, Central Drug Research Institute, Lucknow 226 001.
2. Department of Pathology, K.G. M.C., Lucknow, India.

Malaria is a dreaded disease responsible for causing millions of death or mortality every year. In majority of cases several complications generated during *P. falciparum* infection have been responsible for causing cerebral malaria, which remains still a global challenge to all. Scantly studies are related to changes in malaria infection owing to difficulty in obtaining human cerebral tissue of persons affected due to malaria infection. Therefore, investigations have been carried out with the rodent host harbouring the corresponding parasite. The present studies have been aimed in isolation, characterization and transport of amino acid in isolated cerebral microvessels of normal and *P. yoelii* infected mice. The microvessels were isolated and characterized electron microscopically and biochemically. The biochemical markers (alkaline phosphatase, monoamine oxidase and y-glutamyl transpeptidase) depicted the purity of the preparation. The microvessels thus obtained from normal and *P. yoelii* infected mice were subjected to uptake studies. The data obtained showed that uptake of ^{14}C-glycine was significantly enhanced in infected microvessels as compared to that of normal microvessels. Further, kinetic studies (Km and Vmax) etc. showed that uptake of amino acid was time, temperature and concentration dependent indicating that malaria infection impaired the permeability of blood-brain-barrier unlike other complications.

PS - 10.4

STATUS OF OXIDATIVE STRESS AND ANTIOXIDATIVE DEFENCE SYSTEM IN LYMPHOID TISSUES OF GOLDEN HAMSTERS DURING *LEISHMANIA DONOVANI* INFECTION.

N Goyal, N Mittal and A K Rastogi
Division of Biochemistry, Central Drug Research Institute, Lucknow 226 001

Leishmania donovani, an obligate intracellular parasite residing in macrophages affects the lymphoid system of the host. Macrophages, in an activated state are endowed with the capacity to generate reactive oxygen species (ROS) resulting in antimicrobial activity. Survival of the parasite inside the macrophage and the defence mechanism evolved by the host to cope up the infection is poorly understood. The present study was undertaken to see the changes in the status of oxidative stress and antioxidant system in lym-

phoid tissue during *L. donvan* infection. The results show that significant changes take place in ROS generating and scavenging system in host tissues during leishmanial infection. However, the overall impact of these changes seems to conclude in maintaining lower concentration of ROS *in situ* that can be taken care by the parasite antioxidant system and provide a favourable atmosphere to the parasite to survive.

PS - 10.5

GENOTYPING OF THE P FALCIPARUM INDIAN ISOLATES WITH RESPECT TO MSP 1 PROTEIN

P V Lalitha, Pawan Malhotra, Pawan Sharma and V S Chauhan
International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India

The merozoite surface antigen (MSP-1) , 195 kDa glycoprotein of Plasmodium falciparum undergoes proteolytic cleavage giving rise to a number of fragments.Of these, the carboxyl terminal 19 kDa fragment which is retained on the merozoite surface during invasion is highly conserved with two EGF like domains. It represents a potentially protective vaccine candidate against asexual blood stages of the parasite. In order to design MSP -1 based immunogens relevant for application in different geographical regions, it is of interest to determine microheterogeneity to see the extent of variation in C-terminal region of MSP -1 among isolates from different geographical locations. In facr, such heterogeneity in structure has already been reported from other parts of the world. India being malaria endemic area, we consider it important to see the extent of variation in C-terminal region of MSP-1 among differemt Indian isolates. Here we report our findings in the gene sequences of C-terminus of MSA-1 from isolates collected from three different geographical location in India . Our results show that there exists dimorphism among different isolates of P.falciparum in India with respect to MSP-1. This results is in agreement with the findings of others on MSP-1 from other geographical region in the world.

PS - 10.6

STUDIES ON GST DURING *PLASMODIUM YOELII* INFECTION IN MICE.

Anita Shukla and V C Pandey
Division of Biochemistry, Central Drug Research Institute, Lucknow - 226 001

Malaria is a serious endemic disease in many parts of the world, affecting 40% of world's population. Approximately 2100 million people are at risk of developing the disease annually and some 270 million are infected each year. Glutathione-s-transferase (GST) is a family of widely distributed multifunctional protein that catalyses the conjugation of reduced glutathione (GSH) with various electrophilic substrate. GST can also bind a wide range of hydrophobic non-substrate ligand in a nonconvalent fashion and facilitate their intracellular transportation. The catalytic and transport properties of GST enable the protein to neutralize both endogenous and exogenous toxic substances. So we stud-

ied this enzyme in hepatic tissue of normal and *P. yoelii* infected mice. Kinetic properties of this enzyme were studied in terms of storage at various temperatures. The determination of Km & Vmax of hepatic GST of normal and *P. yoelii* infected mice with GSH as substrate at different concentrations showed that the former had low Km & Vmax as compared to the latter. This maximum activity was obtained in 40-60% fraction. Further purification by column chromatography is in progress to characterize the enzyme and change in isoenzyme profile using affinity chromatography.

PS - 10.7

PARTIAL PURIFICATION AND CHARACTERIZATION OF METHEMOGLOBIN REDUCTASE FROM NORMAL AND *PLASMODIUM YOELII* INFECTED MICE ERYTHROCYTES

Sandhya Srivastava, S K Puri and V C Pandey
Division of Biochemistry and Microbiology, Central Drug Research Institute, Lucknow 226 001.

The pyridine nucleotide dependent methaemoglobin reductase (E.C. 1.6.2.2) is responsible for maintaining haemoglobin in its reduced active state. The activity of this enzyme has been known to be significantly decreased during malaria infection, resulting in increased accumulation of methaemoglobin in the blood. Elevated level of methaemoglobin impairs the normal physiological functions of haemoglobin.

The present study deals with the partial purification and biochemical characterization of methaemoglobin reductase from normal and *P.yoelii* infected mice erythrocytes. The enzyme was purified by ammonium sulfate fractionation and ion exchange chromatography on DEAE - cellulose column. The final preparation of normal and infected sample was purified to about 150 and 132.9 fold over the crude enzyme respectively. The normal purified enzyme was found to be stable up to 168 hrs at 4 ± 1 and -20 ± 1°C. However, the infected purified enzyme was stable up to 72 hrs at 4 ±1 and -20 ± 1°C and after 168 hrs about 20% decrease in the enzyme activity was noticed. The partially purified enzyme (both normal and infected) showed the pH optima in the range of 4.5 - 5.0 and the temperature optima at 35°C. The apparent Km of the enzyme for methaemoglobin was found to be 9.09 and 26 µM for normal and infected sample respectively. p-Chloromercurobenzoate was found to be the specific inhibitor of the enzyme. Primaquine at 150 µM concentration exhibited 46% and 60% inhibition in the enzyme activity of normal and infected mice, respectively. However, no significant inhibition was noticed with chloroquine.

PS - 11.1

MOSQUITO BREEDING ASSOCIATED WITH URBAN SEWAGE SYSTEM

S Haq[1], Rajnikant[2] and V P Sharma[3]
1. Malaria Research Centre, Civil Hospital, Nadiad-387 001.
2. Indian Council of Medical Research, Ansari Nagar, New Delhi.
3. Malaria Research Centre, 22-Sham Nath Marg, Delhi, India

A case study on mosquito problem association with urban sewage treatment plant site of Anand city was carried out during 1994-95. The study revealed that both anopheline and culicine mosquitoes breed in more or less equal proportions throughout the year. The mosquito larval density varied between 10-40/dip, the maximum being recorded during the monsoon months. The mosquito composition of the study area comprised of anophelines (67%), culicines (32%) and *Aedes* species (0.11%). *An. subpictus* (60%) was found to be predominant anopheline species followed by *An. barbirostris* (3.15%) and urban malaria vector *An. stephensi* (0.11%). Among culicine *Cx. vishnui* (15.7%) was the most dominant due to infestation of aquatic vegetation such as water hyacinth. Chemical analysis of water samples revealed high organic contents which generally support the breeding of culicine mosquitoes. Biological control methods using larvivorous fishes, biocides and certain environmental methods have been suggested for the control of mosquito breeding in such habitats.

PS - 11.2

VECTOR CONTROL THROUGH SIMPLE, ECO-FRIENDLY MEASURES IN A PHC SETUP - AN EXPERIMENT AND POTENTIALS

SEWA- Rural Team
A Voluntary Organisation for Rural Health and Development, Jhagadia, Bharuch district, Gujarat.

In the face of diminishing returns in the fight to control malaria and other mosquito-borne diseases, it seems essential to go back to the basics and control the breeding of mosquitoes, through simple environment-friendly measures. In order to test the efficacy and feasibility of such an approach through a PHC setup, the organisation launched a campaign of mosquito control in one subcentre area of Jhagadia PHC. Simple engineering measures were used to eradicate public breeding places, and extensive use was made of larvicidal fish. Kerosene and oil were used, but insecticides of all kinds were totally avoided. Village panchayats and school children were involved wherever possible, although most of the work was done by the male MPHW. Intensive education awareness about mosquitoes and malaria helped in keeping people interested. This intervention continued for a period of two years, and the situation was monitored by measurements of mosquito and vector density using standard entomological procedures. In a control area of similar size and description, vector density was similarly monitored, without institution of any special vector control measures. Almost throughout the study

period, vector density remained below the critical level, whereas it was far above the critical in the control area all the time. The organisation then launched a campaign in a large village to achieve vector control through community participation. The experiences and results of both these experiments will be presented and potentials for such interventions will be discussed.

PS - 11.3

A R Singh

(Abstract not received)

PS - 12.1

PARASITISM INDUCED CHANGES OF HEMOLYMPH PROTEINS IN MALARIA VECTOR *ANOPHELES STEPHENSI* (DIPTERA : INSECTA)

S K Gakhar and Harish Shandilya
Department of Biosciences, M.D. University, Rohatak-124001

The changes in hemolymph proteins pattern during the development and ageing of Anopheles stephensi due to Parasitism by *Plasmodium yoelii yoelii* was analysed by SDS-PAGE. In addition, the changes in the glucose feeding and sex specific proteins have also been studied.

One polypeptide (26KDa) was commonly induced by parasite infection in the hymolymph during young and old age. In addition, 7 polypeptides (16.5, 46, 48, 50, 55, 76 & 83 KDa) wer also synthesized after 9-days of parasitized blood meal. Only one polypeptide (31 KDa) disappeared due to parasite invasion in ageing mosquitoes. The appearance due to parasite invasion in ageing mosquitoes. The appearance of polypeptides could protect the parasite and also facilitate transport from gut to gland and/or out of the gland during feeding on the host.

Nine polypeptides (30, 102, 110, 118, 122, 140, 150, 155 & 160 KDa) generally disappeared when the females were maintained exclusively on glucose on different days of adult life. However, three polypeptides (16.5, 55 and 158 KDa) disappeared only during senescence. On the contrary many polypeptides were induced due to glucose feeding viz. 25, 29, 36, 50, 57 & 76 KDa on various days of adult life.

Regarding sex specific proteins, Five polypeptides were found exclusively in female hymolymph and 3 polypeoptides in the males. The spatial and sex specific differences of these polypeptides in hemolymph most likely reflects differences in the feeding capabilities of two sexes.

PS - 12.2

IMPACT OF PRIOR BLOOD MEALS ON THE SUSCEPTIBILITY OF *ANOPHELES STEPHENSI* TO *PLASMODIUM VIVAX* MALARIA INFECTION

O P Singh, T Adak and V P Sharma
Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.

The malaria vectors in nature may take several blood meals before contacting an infective host. In order to know the effect of prior blood meals on the susceptibility of vector to malaria parasite, the comparative susceptibility of prior blood fed and unfed *An. stephensi* to *P. vivax* infection was examined. A cohort of laboratory reared *An. stephensi* were divided into three batches : first batch was fed on uninfected blood meals twice, second batch was fed on blood meal once and third batch was fed on glucose only which

served as control. Mosquitoes of all the batches were later fed simultaneously on *P. vivax* infected blood through artificial membrane. Examination of midgut revealed higher oocyst rate in mosquito which had taken two prior blood meals (96.71%) and one prior blood meal (61.36%) as compared to the control (10.32%) indicating that prior blood meals taken by *An. stephensi* enhance their susceptibility to *P.vivax* sporogony.

PS - 12.3

HEME BIOSYNTHISIS BY THE MALARIAL PARASITE : IMPORT OF - AMINOLEVULINATE DEHYDRASE FROM THE HOST RED CELL

Zahid Q Bonday and G Padmanaben
Department of Biochemistry, Indian Institute of Science, Bangalore - 560 012, India

The mouse and human malarial parasites, *plasmodium berghei* and *Plasmodium falciparum* respectively, synthesize heme *de novo* following the standard pathway observed in animals despite the availability of large amounts of heme, derived from red cell hemoglobin, which is stored as hemozoin pigment. The enzymes, - aminolevulinate dehydrase (ALDA), coproporphyrinogen oxidase (CPO) and feerochelatase (FC) are present at strikingly high livels in the *P.berghei* infected mouse red cell *in vivo*. The isolated parasite has low levels of ALAD and the data clearly indicate it to be of red cell origin. The purified enzyme preparations from the uninfected red cell and the parasite are identical in kinetic properties, subunit molecular weight, cross-reaction with antibodies to the human enzyme and N-terminal aninoacid sequence. Immunogold electron microscopy of the infected culture indicates that the enzyme is present inside the parasite and therefore, is not a contaminant. The parasite derives functional ALDA from the host and the enzyme binds specifically to isolated parasite membrane *in vivo* suggestive of the involvement of a receptor in its translocation into the parasite. While, ALID, CPO and FC from the parasite and the uninfected red cell supematant have identical subunit molecular weights on SDS-PAGE and show immunological cross-reaction with antibodies to the human enzymes, as revealed by western analysis, the first enzyme of the pathway, namely, -aminolevulinate systhase (ALAS) in the parasite, unlike that of the red cell host, does not cross react with antibodies to othe human enzyme. However, ALAS enzyme activity in the parasite is higher than that of the infected red cell supernatant. It is, therefore, concluded that the parasite, while making its own ALAS, imports ALAD and perhaps most jof the other enzymes of the pathway from the host to synthesize heme *de novo* and this would enable it to seggregate this heme from the heme derived from red cell hemoglobin degradation. ALAS of the parasite and the receptor(s) involved in the translocation of the host enzymes into the parasite would be unique drug targets.

PS - 12.4

TRANSMISSION REDUCING FACTORS IN CAMEROONIAN GAMETOCYTE CARRIERS

L. Mulder, J.P. Verhave and R.W. Sauerwein
Med. Parasitology, University Nijmegen, The Netherlands; OCEAC Yaounde, Cameroon

Experimental infections were carried out with blood of gametocyte carriers, to identify natural transmission-reducing (TR) factors. Replacement of OWN plasma by AB plasma from non-immunes resulted in significant increase of mosquito-infection. Plasma factor(s) can thus reduce the transmission capacity of gametocyte carriers to mosquitoes. The replacement procedure was improved by washing the parasitized erythrocytes with warm RPMI. The TR capacity of gametocyte carriers was compared to the capacity of their serum to block the infectivity of cultured parasites, *P. falciparum* NF54 strain. For the Yaounde experiments the TR was expressed as : TR = (100 - 100 * OWN / AB). TR was determined by comparing proportions of infected mosquitoes. In Nijmegen TR was assessed by comparing the oocyst numbers in the serum batch compared to the numbers in mosquitoes fed on control serum. TR was defined as a percentage of reduction between 30 and 90%. TB was considered as a percentage of reduction of at least 90%. Results of the Yaounde assay showed that 32.8% of the sera blocked significantly. The Nijmegen bio-assay showed that 22 of 48 sera (37.9%) produced TR over 30% with 6 sera over 90%. There was a significant correlation between the result of both assays with the sera from gametocyte carriers from Cameroon ($r=0.41$, $p<0.05$). The majority of the blockers in Nijmegen did not (or hardly) give infection in the Yaounde bio-assay. The antibody tests for sexual stage antibodies were correlated with TR. Mabs anti-Pfs48/45 were used to develop a competition ELISA (cELISA), to detect natural Pfs48/45 antibodies. Agreement between cELISA using 5 epitopes and blocking activity in Cameroonian gametocyte carriers was about 75%.

Only 7% of individuals were positive in an area with high transmission (250/pp/year), with titres ranging from 1/20 to 1/320, while in an area with 25 bites/pp/year, 3% were positive, with titres of 1/20. Contrarily, some 66% of sera from Cameroonian gametocyte carriers was positive; apparently the anti-gamete antibodies are short lived.

PS - 12.5

ISOLATION OF *PLASMODIUM VIVAX* REFRACTORY STRAINS OF *ANOPHELES CULICIFACIES* SPECIES B

T Adak, Sarbjeet Kaur, O P Singh and V P Sharma
Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.

One of the possible approaches for genetic control of vector species is replacement of vector population with refractory strain either selected in laboratory or manipulated through transgenic technology. No refractory anopheline strain in vector species has

been identified so far which is completely refractory to human malaria parasite. We report here for the first time isolation of two *An. culicifacies* species B strains that are refractory of *P. vivax* sporogonic development.

An. culicifacies sensu lato populations from different parts of the country were screened cytologically, and pure colonies of *An. culicifacies* species A, B and C and their polymorphic forms with respect to Y chromosome (acrocentric or sub-metacentric) were established in laboratory. Their susceptibility to *P. vivax* infection was checked by feeding mosquitoes through artificial membrane on *P. vivax* infected blood. We succeeded in isolating two refractory strains of *An. culicifacies* acrocentric species B. In all the feeding experiments with 22 *P. vivax* isolates, late ookinete stages were found encapsulated by thick melanin forming structures of varying shapes and sizes in the midgut epithelium without any further sporogony. The molecular and genetic basis of refractoriness of the two strains would be discussed.

PS - 13.1

RESISTANCE REVERSAL POTENTIAL OF QUINIDINE AGAINST CHLOROQUINE RESISTANCE OF *PLASMODIUM YOELII NIGERIENSIS* (MDR STRAIN)

M Kazim[1], Aseem Umesh[1], W K Milhous[2], A Krishan[3] and G P Dutta[1]
1. Division of Microbiology, Central Drug Research Institute, Lucknow
2. Dept. of Experimental Therapeutic, WRAIR, Washington.
3. Dept. of Radiation Oncology, Division of Experimental Therapeutics, P.O. Box 016960, Miami, Florida 33101, USA.

Quinidine had been reported earlier to exhibit antimalarial activity against both sensitive and chloroquine/mefloquine/quinine resistant strains of *P. berghei* and *P. yoelii nigeriensis* (MDR). In areas of chloroquine resistance *P.falciparum* the efficacy of quinidine has also been proved clinically by several investigators. Recent studies on tumor-cell resistance have suggested that quinidine had an additional property of blocking drug efflux of anti-cancer drugs, thereby enhancing cytotoxic drug retention and consequently reducing drug resistance in advance cancer patients. The resistance reversal effect of quinidine against chloroquine resistance of *P. yoelii nigeriensis* (MDR) was demonstrable when the low dose quinidine (10-25 mg/kgx4) was given together with chloroquine (4-8 mg/kgx4) in post-infection schedule (from day +3 to +6) as shown by nearly 50% survival of treated mice beyond 28 days, while there was no survival when individual drugs (chloroquine or quinidine) alone were given. The mean survival time of Swiss mice for chloroquine alone treatment was 12.66 - 13.16 days and quinidine alone treatment 11.83 - 13.83 days, while the MST of chloroquine plus quinidine treatment ranged between 23.83 - 27.0 days. This study suggests that quinidine in combination with chloroquine exerts resistance reversal action on the MDR parasite *in vivo* in mouse model.

PS - 13.2

CHEMOPROPHYLACTIC AND RESISTANCE MODULATING POTENTIAL OF ANTIHISTAMINIC AGENTS AGAINST *PLASMODIUM YOELII* IN SWISS MICE.

Naresh Singh, S.K. Puri, W.K. Milhous* and G.P. Dutta
Division of Microbiology, C.D.R.I., Lucknow, India.
*Experimental Therapeutics Division, WRAIR, Washington, DC, USA.

The spread of drug resistant malaria has necessitated identification of alternate agents and strategies for chemotherapy and chemoprophylaxis. Experimental studies using *P.yoelii nigeriensis* (N-67))/ Swiss mice rodent model have shown promising casual prophylactic as well as resistance modulating potential of some of the known tricyclic compounds which are currently being used as anti-histaminic (H1-receptor antagonists) drugs. Treatment with ketotifen and cyproheptadine at 5 mg/kg as well as with terfenadine

at 50 mg/kg for three days (-1,0, +1) protected mice against challenge with *P.yoelii* sporozoites. Azatadine and loratadine at 10 mg/kg showed partial protection.

The resistance modulating potential of antihistaminic drugs was evaluated using experimentally selected isolates resistant to maximum tolerated doses of chloroquine, mefloquine, halofantrine or pyrimethamine. The response was monitored after administration of antihistaminic drugs in combination with one of the antimalarials. The results showed that cyproheptadine (10mg/kg) lowered the curative doses of chloroquine, mefloquine and halofantrine against respective resistant strains to the level effective against the parent sensitive strain. The resistance modulating effect of other agents in decreasing order was ketotifen > pheniramine > azatadine> loratadine > terfenadine. The agents however, did not alter the sensitivity of pyrimethamine against pyrimethamine resistant strain. The study provides evidence to exploit the chemoprophylactic and therapeutic potential of antihistaminic agents for management of drug resistance in malaria.

PS - 13.3

EVALUATION ANTILEISHMANIAL ACTIVITY OF SOME MEDICINAL PLANTS

Alema Zaidi, P Y Guru, D K Kulshreshtha[1] and J C Katiyar
Division of Parasitology and * Medicinal Chemistry, Central Drug Research Institute, Lucknow-226 001, India.
1. Division of Medicinal Chemistry, CDRI, Lucknow-226 001, India.

Kala-azar is a major public health problem. Chemotherapy is the only practical mean to control the disease. Partial effectiveness of conventional drugs (Stibanate, Pentamidine & Amphotericin-B) in certain cases added with the toxic side effects have made the situation more demanding for new antileishmanials. Recently, there has been major emphasis to explore the possible antileishmanial properties of terrestrial plants which are likely to possess less toxic side effects. In accordance certain plants known to possess medicinal value in traditional remedies, were screened for antileishmanial activity. A total of 58 alcoholic extracts of various plants were tested against established infection of *L. donovani* in golden hamster. These extracts were administered orally at the dose level of 1g/kg for 5 consecutive days. The efficacy assessment was made on day 7 post treatment in spleen dab smears. Of the various extracts tested, only three viz CDRI code no. 4021, 4023, 4073 produced > 70% inhibition of amastigote multicaplication. Certain other (CDRI code no. 4015, 4194, 4224, 4230, 4234) exhibited mild antileishmanial activity. The 3 extracts (> 70% activity) are under elaborate investigation to single out the most effective one for further developmental work and isolation of active principle(s).

PS - 13.4

BRUGIA MALAYI IN INDIAN LANGUR (*PRESBYTIS ENTELLUS*) : ALTERATIONS IN ANTIGEN RECOGNITION PATTERN AFTER DIETHYLCARBAMAZINE (DEC) TREATMENT

K Tyagi, P K Murthy and R K Chatterjee
Division of Parasitology, Central Drug Research Institute, Lucknow - 226 001, India.

DEC is the drug of choice for lymphatic filariasis and is known to alter both antibody and cell mediated immune responses of the host. Of the various experimental models used Indian langurs infected with *B. malayi* have been found to mimic human situations especially in the development of pathological symptoms. The present study has been carried out to observe the alterations in antigen recognition pattern of *B. malayi* infected langurs after DEC treatment. Langurs having 90 days old infection with progressive rise in circulating microfilariae (mf) were treated with DEC (200 mg/kgx5, p.o). Serum samples were collected before (i.e. day 'O') and after (day +8, +30 & +80) the commencement of the treatment. *B. malayi* adult worm antigen fractionated by SDS-PAGE and transferred onto nitrocellulose (NC) sheets was identified by IgG antibodies present in the sera samples. It was observed that strong reactivity of the functional molecules became faint after DEC treatment. Intensity of these molecules further diminished 80 days post therapy. On the other hand, 96 and > 53 kDa molecules which were not recognised earlier, appeared in blots post therapy only. The appearance and disappearance of immunoreactive bonds seem to be related to the death/elimination of mf and/or adult filariids. The significance of alterations in the immunoreactivity of treated langur sera would be discussed.

PS - 13.5

EFFICACY OF HUMAN β - CASEIN ANALOGUE (CDRI COMP. 89/215) AN IMMUNOPOTENTIATOR, AS AN ANTILEISHMANIAL AGENT AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS

Anuradha, P Sharma, W Haq and J C Katiyar
Div. of Parasitology and Biopolymers, Central Drug Research Institute, Lucknow-226 001, India.

The profound impairment of immune system of the infected host, a characteristic feature of visceral leishmaniasis, contributes significantly to the partial success of chemotherapy. Since, in leishmaniasis, cure is the combinatorial effect of drug and immunity, the rationale approach towards antileishmanial chemotherapy would be to potentiate the immune functioning of the host to extract desired results. To meet this end, several rationally designed analogues of human β – casein fragments were evaluated for their ability to stimulate the nonspecific resistance of hamsters against *Leishmania donovani* infection. Among them Val-Glu-Gly-Ile-Pro-Tyr (Comp. 89/215) showed considerable prophylactic property both *in vivo* and *in vitro*. Its usefulness in therapeutic efficacy

was evaluated in established infections of *L. donovani* at different dose schedules and by different routes. The compound exerted considerably good therapeutic efficacy by parenteral route (i.p.). No effect was, however, observed by oral route. The optimum antileishmanial efficacy (62% inhibition of parasite multiplication was noticed at a dose of 1mg/kg given daily for 5 days. Lower dose of 0.5 mg/kgx5 was ineffective.

This compound with its potentiality of stimulating nonspecific immune status together with antileishmanial activity may provide a novel lead towards antileishmanial chemotherapy.

PS - 13.6

EVALUATION OF ANTIFILARIAL ACTIVITY OF PLANT PRODUCTS USING *IN VITRO* AND *IN VIVO* SCREENING METHODS

Sunita Bhatnagar and R K Chatterjee
Parasitology Division, Central Drug Research Institute, Lucknow-1

Lymphatic filariasis is a chronic disease prevailing in tropical and subtropical countries. The drug available for this disease is chiefly microfilaricidal. In absence of any effective chemical lead for chemotherapeutic attack against adult filariids, it was thought for worth while to identify some active agents from terrestrial plants having the history of their use in traditional medicines or otherwise. In the present study, 18 such plant extracts were tested for antifilarial activity using *in vitro* and *in vivo* test systems. In *in vitro* test two parameters eg. motility test and MTT reduction assay were used whereas in *in vivo* assay, *Mastomys coucha* infected with *Acanthocheilonema viteae* was used. Of the 18 extracts plant nos. 3929, 3942, 3957, 3961, 3973 and 3938 were active *in vitro* but failed to exert any effect in *in vivo* system. Plant numbers 3997, 3966, 3975, 3925, 4029, 3986, 3968, 3989 and 3959 showed activity in both *in vitro* and *in vivo* systems. Rest of the plant materials were inactive in both screening systems. Thus, percent false positive in *in vitro* test was 40% whereas there was no false negative case. Thus, a good percentage of compounds showed correlation between *in vitro* and *in vivo* test systems suggesting adoption of *in vitro* systems in the primary screening of natural products.

PS - 13.7

A NOVEL ACTIVITY OF POLY ICLC AN INTERFERON INDUCER CAUSING INTERRUPTION OF MALARIA TRANSMISSION

Renu Tripathi[1], R K Maheshwari[1], Hilton B Levy 3 and G P Dutta[2]
1. Division of Microbiology, C.D.R.I., Lucknow, India.
2. Uniformed Services University of Health Sciences, Bethesda, MD, USA.
3. National Institute of Health, Bethesda, MD USA.

In view of the serious threat of transmission of drug resistant malaria, global efforts are continuing to develop a strategy for the control of malaria transmission through either

the development of transmission blocking vaccine or the development of a safe and effective gametocytocidal drug which could effectively replace primaquine, the currently available gametocytocide in clinical use. The present communication reports first use of Poly ICLC as a gametocytocide. Poly ICLC an inducer of interferon has been found to interrupt the simian malaria transmission. This cytokine was studied using *P.cynomolgi* B infected rhesus monkeys transmission model and using colony bred *Anopheles stephensi* as vector. Normally gametocyte carrying rhesus monkeys maintain infectivity for *Anopheles stephensi* for several days but the administration of Poly ICLC was found to completely block the infectivity within 18 hrs of treatment. Individual mosquito dissections showed that Poly ICLC interrupts the oocyst formation as well as sporozoite development in *An. stephensi* resulting in complete loss of mosquito infectivity.

PS - 13.8

STUDIES ON AETIOLOGY OF REACTOGENECITY OF DIETHYLCARBAMAZINE IN EXPERIMENTAL MICROFILARAEMIC HOST

Nigar Fatma[1], V M L Srivastava[2] and R.K. Chatterjee[1]
1. Division of Parasitology, Central Drug Research Institute, Lucknow-226 001.
2. Division of Biochemistry, CDRI, Lucknow-226 001

Reactogenecity of DEC is very common in microfilaraemic patients and the intensity of reaction is proportional to the concentration of mf in peripheral blood. Such reactions occur in filaria infected animals also. In the present study, investigation was made to understand physiologic disturbances in *Mastomys coucha* with *Acanthocheilonema viteae* infection following DEC treatment. In this model, DEC caused reactogenecity leading to death of animals. It was interesting to note that the same host with *Litomosoides carinii,* a metabolically facultative parasite, did not reveal such reaction. In both the cases, mf were trapped in the liver. In comparative biochemical studies, the level of transaminases, cholesterol, bilirubin and alkaline phosphatase in serum were very high in former model (*A. viteae*/Mastomys) showing disturbances in longer function while in the latter system (*L. carinii*/Mastomys), these parameters remained almost unchanged. Nevertheless, the concentration of NO and H_2O_2 in the liver of Mastomys bearing either of the infections were significantly higher of mastomys bearing either of the infections were significantly higher after DEC -therapy. Both these metabolities (NO and H_2O_2) were highly cytotoxic and known to mediate the killing of microfilariae. Therefore, killing of both the parasites appear to occur by the similar mechanism. However, side reactions as observed in Mastomys with *A. viteae* infection appear to be dependent on the substances (eg Ags) released by the killed mf and also on the host sensitivity to these substances. Investigation of the nature of such substances is in progress.

PS - 13.9

EXPERIMENTAL STUDIES WITH QUININE-TETRACYCLINE VERSUS QUINIDINE - TETRACY CLINE COMBINATION AGAINST MULTIDRUG RESISTANT *PLASMODIUM YOELII NIGERIENSIS* INFECTION IN SWISS MICE.

Aseem Umesh, M. Kazim, S K Puri, W K Milhous* & G P Dutta
Division of Microbiology, C.D.R.I., Lucknow.
* Walter Reed Army Institute, Washington, D.C. USA.

The spread of *P.falciparum* resistant to chloroquine and other newer antimalarial drugs is hindering the success of global malaria eradication programmes. Amongst all the known antimalarials, quinine had been in effective use for longest period, although its utility is now threatened because of decreased sensitivity in certain parts. Quinine-tetracycline combination has been extensively used in South East Asia during the last two decades. In the present study efficacy of various regimens of quinine-tetracycline and quinidine-tetracycline combinations, has been compared against multidrug resistant rodent model. A multidrug resistant strain of *Plasmodium yoelii nigeriensis* exhibiting resistance to maximum tolerated doses of chloroquine mefloquine, a modiaquine, quinine, quinidine and halofantrine was employed for the study. Results showed that seven dose treatment (Day 0-6) with quinidine 400mg/kg plus tetracycline 50mg/kg/ doxycycline 25mg/kg was curative, as it prevented development of patent infection in all the treated mice. On the other hand, quinine 400mg/kg plus tetracycline 50mg/kg/ doxycyline 25mg/kg combinations in the same regimen provided partial protection. Likewise, quinidine 300mg/kg plus tetracycline 250mg/kg combination provided 100% protection while 300mg/kg plus tetracycline 250mg/kg protected 62.5% of the treated mice. The study concludes that quinidine-antibiotic combination may show better efficacy against multi drug resistant *Plasmodium falciparum* strains.

PS - 13.10

BIOCHEMICAL MECHANISM FOR CHLOROQUINE RESISTANCE REVERSAL AND ANTIMALARIAL ACTION OF CYPROHEPTADINE AGAINSNT MULTI DRUG RESISTANT *PLASMODIUM YOELII NIGERIENSIS*

Rashmi Agrawal[1], B.L. Tekwani[2], Renu Tripathi[1], O.P. Shukla[2] and G.P. Dutta[1]
Divisions of Microbiology[1] and Biochemistry[2], Central Drug Research Institute, Lucknow-226 001 (INDIA)

Emergence of drug resistant strains of malaria parasites is the leading cause of resurgence of the disease. There is an urgent need for development of the drugs effective against resistant strains or identification of the agents which may modulate the resistance property of malaria parasite. Several classes of the compunds viz., calcium channel blokers, antidepressants and antihistaminics have been identified as potent resistance reversal agents. Among these, the antihistaminics have been identified as potent resistance reversal agents. Among these, the antihistaminics like cyproheptadine have

also been found to possess inherent antimalarial activity. In the present study, a multi drug resistant strain of *Plasmodium yoelii nigeriensis* which shows resistance to chloroquine (CQ), mefloquine and quinine was used. The antimalarial action of cyproheptadine *in vivo* againstt *P. yoelii* was recorded at the dose as low as 10 mg/kg body wt. of mice in a 4 day oral schedule and complete protection was observed at the dose of 20-25 mg/kg body wt. Cyproheptadine in combination with CQ showed significant protection even at lower doses. Desipramine and verapamil, the standard resistance reversal agents, were inactive *in vivo* even up to the dose of 30 and 50 mg/kg through its interaction with the haemozoin formation pathway. Malaria parasite degrades host cell haemoglobin to fulfill its nutritional requirements and the resulting free haem is detoxified by its polymerisation to a crystalline, black brown pigment known as 'haemozoin'. CQ alone did not cause noticeable effect on haemozon content of intact *P. yoelii* infected erythrocytes *in vitro*. However, cyproheptadine alone caused considerable decrease in the level of haemozoin. The effect was much more prominent when a combination of CQ and cyproheptadine was used. Cyprohptadine also caused dose dependent inhibition of haem polymerase activity of *P. yoelii* extracts. Desipramine and verapamil do no show any effect on malarial haem polymerase. Inhibition of haem detoxification function of the parasite may be proposed as a mechanism of antimalarial action of cyproheptadine.

PS - 13.11

SYNERGISTIC EFFECT OF ARTEETHER AND POLY ICLC TREATMENT ON HEPATIC OXIDATIVE STRESS AND ANTIOXIDANT DEFENCE INDICES DURING *PLASMODIUM YOELII NIGERIENSIS* INFECTION IN MICE

Nikhat J Siddiqqi[1], S. K. Puri[1], R.K. Maheshwari[2], G.P. Dutta[1] and V C Pandey
1. Division of Biochemistry and Microbiology, Central Drug Research Institute, Lucknow 226 001, India
2. Uniformed Services University of the Health Sciences, Bethesda, Maryland, U.S.A.

Plasmodium yoelii nigeriensis produces lethal infection in Swiss albino mice. Reactive oxygen species such as superoxide anions and hydrogen peroxide have been implicated in the pathogenesis of malaria. *P. yoelii nigeriensis* infection in mice resulted in an increased generation of hepatic oxidative stress indice *viz*.. xanthine oxidase and rate of lipid peroxidation. There was also an increase in the antioxidant defence system represented by reduced glutathione and glutathione reductase but a decrease in superoxide dismutase and catalase.

Arteether treatment of infected mice caused a significant decrease in hepatic xanthine oxidase and rate of lipid peroxidation but on significant change in the antioxidant defence system. However, combination therapy of arteether with poly ICLC (an interferon inducer) to infected mice resulted in the restoration of all the hepatic oxidative stress and antioxidant defence indices to near normal levels. These results suggest that

combination of arteether with poly ICLC is a better antimalarial treatment than arteether alone.

PS - 13.12

KETOCONAZOLE REVERSES MEFLOQUINE RESISTANCE OF MULTIDRUG RESISTANT *PLASMODIUM YOELII NIGERIENSIS*

Aradhana Awasthi[1], Vinod Bhakuni[1], Renu Tripathi[1], W Milhous[2] and G.P. Dutta[1]
1. Division of Microbiology and Division of Membrane Biology, C.D.R.I., Lucknow.
2. Dept. of Exptl. Therapeutics, WRAIR, Washington, D.C.

The resurgence of malaria is posing serious public health problem during the last two decades and in South-East Asia particularly, the mefloquine resistant areas are gradually expanding. In the present study we have assessed the possible use of ketoconazole (a cytochrome P-450 inhibitor) in reversal of mefloquine resistance of MDR strain of *P.yoelii nigeriensis*. Mice infected with *P.y. nigeriensis* were treated with mefloquine (4 and 8mg/kg) together with ketoconazole (0.625, 2.5 and 5mg/kg). The ketoconazole has no apparent anti-malarial effect and doses of 0.625, 2.5 and 5mg/kgx4 resulted in MST of (8.5 to 8.66 days respectively) in comparison to the control groups which survived for 7.33 - 8.33 days. Since the MDR strain is resistant to mefloquine, doses of 4.0 and 8mg/kg of mefloquine slightly extended the MST to 8.33 and 14.33 days respectively. The results of the resistance reversal study that ketoconazole (5mg/kgx 4 days) which has no antimalarial effect, when given together with mefloquine as shown by increased suppression of parasitaemia between 8 and12 days of infection, leading to significant extension of MST to 16.66-21 days in the combination treatment group, whereas survival of mefloquine groups was 11 to 13 days and that of ketoconazole group 8.5 to 9.5 days. To find out the mechanism of reversal of drug resistance, the ketoconazole and mefloquine groups when given additional dose of phenobarbitone (5mg/kgx4 days) abolished the suppression of parasitaemia and the MST was also reduced to 12-13.5 days.

PS - 13.13

COMPARATIVE ANTIMALARIAL PROFILE OF α, β AND α/β ARTEMETHER AGAINST MULTI-DRUG RESISTANT MURINE MALARIA.

Kavita Purbey, Chandan Singh and S K Puri
Division of Microbiology and Division of Medicinal Chemistry, CDRI, Lucknow, India. Central Drug Research Institute, Lucknow, India.

The discovery of the natural peroxide qinghaosu (artemisinine) as the active principle from the traditional Chinese herbal medicine led to the advent of new antimalarials and several of its derivatives including dihydroartemisinine, artemether, arteether and artesunate have been successfully used in experimental models and in humans. The synthesis of artemether is known to yield two crystalline anomers α and β. We have

compared the antimalarial potency of two enantiomers and the racemic mixture against a multi-drug resistant strain of *P.yoelii nigeriensis* in Swiss mice. A dose of 3 mg/kgx 4 days (Day 0-3) prevented development of patent infection in animals treated with either of the three components via intramuscular route. β and α/β artemether showed comparable curative efficacy after oral administration at 64 mg/kg while similar response with α anomer was obtained at 128 mg/kg dose. ED_{50}/ED_{90} values did not show any significant difference in the activity of two anomers and the mixture. To compare the duration of protection after single dose treatment, groups of mice were administered either of the three components at 64 mg/kg, im and challenged at varying intervals posttreatment. Results showed that β anomer protected all the mice treated on day 0 -1, -2 or -3 and 50% of the animals treated on day-4. The racemic mixture was partially effective in inhibiting the infection in mice treated on day -4, -3 or -2 and protected all the mice treated on day -1, or 0. The α anomer was least effective since only 16% and 66.6% of the mice treated respectively on day -1 or day 0 were protected. The study provides evidence of the varying pharmacodynamic properties of the anomers of artemether.

PS - 13.14

ANTHELMINTIC EFFICACY OF *FLEMINGIA VESTITA* (LEGUMINOCEAE) : GENISTEIN - INDUUCED ALTERATIONS IN THE ACTIVITY OF TEGUMENTAL ENZYMES IN THE CESTODE, *RAILLIETIŃA ECHINOBOTHRIDA*.

P Pal and V Tandon
Department of Zoology, North-Eastern Hill University, Shillong - 793 022, India

The crude root-tuber peel extract of *Flemingia vestita,* an indigenous leguminous plant of Meghalaya, and its active chemical component, i.e., genistein, have shown paralysing effect on soft-bodied helminth parasites of the trematode and cestode groups. Morphological and ultrastructural studies of the *in vitro* treated cestode *Raillietina echinobothrida* revealed alterations in the tegumental architecture. With a view of investigating the mode of action of the plant-derived component, its effect on the activity of tegumental enzymes of the parasite was studied. Acid phosphatase (AcPase), alkaline phosphatase (AlkPase), adenosine triphosphatase (ATPase) and 5' - nucleotidase (5' -Nu) are predominantly distributed in the tegument, subtegument and somatic musculature. After exposure to the crude extract (50 mg/ml of the incubation medium) and genistein (0.5 mg/ml), a pronounced decline in the visible stain intensity was noticeable indicating very less or no activity in these sites. Quantitatively the activity of AcPase, AlkPase, ATPase and 5' -Nu was found to be suppressed by 97%, 95%, 88% and 57%, respectively following genistein treatment. The reference drug, praziquantel (0.01 mg/ml) also caused a reduction in the enzymes' activity, somewhat at par with the genistein treatment. The results suggest that the tegumental enzymes of the parasite may be an important target of action for genistein, which appears to act transtegumentally.

PS - 13.15

PREDICTION OF ANTIMALARIAL POTENCY USING CALCULATED MOLECULAR ELECTRONIC PROPERTIES

Apurba K Bhattacharjee, Tweeny R Kau and Jean M. Karle
Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC, USA

Quantum chemical computations on a series of mefloquine analogs reveal correlations between specific molecular electronic properties and *in vitro* antimalarial activity providing a set of discriminators of antimalarial efficacy. The 3-dimensional structures of 21 mefloquine analogs were optimized using the semi-empirical AMI method. Electronic properties such as dipole moment, molecular electrostatic potentials (MEPs) and highest occupied molecular orbitals (HOMOs) were calculated. The most efficacious compounds have a dipole moment pointing towards the quinoline nitrogen atom. Profiles of negative MEP beyond the Van der Waals surface reveal that a large laterally extended region in the frontal portion of the quinoline ring and an absence of the potential over the aromatic ring for the CF_3-containing compounds are linked to potent activity. The MEP profile at the Van der Waals surface shows that the site for the most positive potential lies by the hydroxyl proton whereas the site for the most negative potential is either by the piperidyl or the quinoline nitrogen atoms. A negative potential in the range - 62 to -55 kcal/mol by the aliphatic nitrogen atom and a positive potential greater than 35 kcal/mol by the hydroxyl proton appear to be two important features for potent activity.Proton affinity of the aliphatic nitrogen atom indicates that a value > 218 kcal/mol does not favour potent activity. HOMO energies are more negative for the more potent compounds containing electron-withdrawing substituents like CF_3 on the quinoline ring. Overall, electron-withdrawing substituents at specific positions and intermolecular hydrogen bond formation appear to be two crucial features responsible for *in vitro* activity whereas increased lipophilicity and nuclephilic susceptibility of the aromatic ring together with intermolecular hydrogen bond formation contribute to *in vivo* activity as reported by us earlier. These observations illustrate the fundamental molecular forces that affect the function and utility of the compounds and therefore, should aid in the designing of new antimalarial compounds.

PS - 13.16

PLASMA CHLOROQUINE AND DESETHYLCHLOROQUINE CONCENTRATIONS IN PATIENTS INFECTED WITH *P.VIVAX* MALARIA AFTER GIVIING STANDARD TREATMENT USING HPLC.

P K Kar[1], V K Dua[1] and V P Sharma[2]
1. Malaria Research Centre, Field Station, BHEL, Ranipur, Hardwar 240 403
2. Malaria Research Centre, 22 Sham Nath Marg, Delhi-110 054 India.

Chloroquine and Desethylchloroquine concentrations were determinined on day 0,1,2,3,4

and 5 after treatment with 900 mg chloroquine base (D0 600; D1 300) to 50 *P. vivax* malaria patients using HPLC method. Acetonitrile-methanol-diethylamine (60 : 10 : 0.5 v/v) at a flow rate of 1.5 ml min^{-1} was pumped on μ porasil (3.9x300mm) normal phase column. Chloroquine and Desethylchloroquine were detected on fluorimetric detection at Ex 340 nm and Em 380nm. The plasma chloroquine concentrations in all cases were found above therapeutically effective concentration of 16 μg/ml. Chloroquine to desethylchloroquine ratio varied between 2-3 times in all cases.

PS - 13.17

SINGLE DOSE BIOASSAY STUDIES WITH HALOFANTRINE IN CHLOROQUINE RESISTANT RODENT MALARIA MODEL

Deepti Agarwal and S K Puri
Division of Microbiology, Central Drug Research Institute, Lucknow.

Halofantrine, a new 9-phenanthrenemethanol antimalarial is selectively active against intraerythrocytic stages of drug-resistant rodent parasites as well as against *P. falciparum* malaria. Halofantrine is an important treatment alternative in the present scenario of escalating parasite resistance to conventional antimalarials and efforts are needed to protect and preserve the longevity of the drug by coadministration with appropriate agents. Experimental studies have been carried out in an innately chloroquine resistant *Plasmodium yoelii* (N-67) Swiss mice model, to determine the kinetics of protection afforded against parasite challenge after single dose treatment. A dose of 8 mg/kg x4 days (Day 0-3) was established as the curative dose in this model, as none of the mice developed patent infection after challenge. For the single dose bioassay, groups of mice were administered single oral dose of halofantrine (16mg/kg or 32mg/kg) and challenged at various intervals post treatment. Results showed that none of the mice administered 32 mg/kg halofantrine developed patent infection when challenged on the day of treatment while partial protection was observed in animals challenged 24 hr or 72 hr after drug administration. None of the mice challenged 120 hr after treatment was protected. Likewise, none of the mice challenged 72 hours post 16 mg/kg dose treatment was protected and this dose protected only 62.5% mice challenged on the day of treatment. The results obtained from the study would provide base line data for developing drug combinations to protect halofantrine.

PS - 13.18

DRUG SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* IN THE SOUTH-EASTERN REGION OF BOLIVAR STATE, VENEZUELA.

A Carballo[1] and A Rodriguez-Acosta [2]

1. Institute of Health, Malariology, Bolivar state and Universided Central de Venezuela.

2.Tropical Medicine Institute, P.O. Box: Apartado 47423 Caracas 1041. Venezuela.

In the zones of malarial transmission at Domingo Sifontes Municipality (I.P.A. in 1994 was 352/1000 inhabitants, 22.7% *Plasmodium falciparum*) Bolivar state, where gold mining is the main activity, diagnosed a number of patients with *P. falciparum* with a determinate amount of the presented clinical early or late symptoms of recrudescence of the signs of malaria disease, producing a delay in the clinical-parasitological improvement and an increase in the morbi-mortality of malaria. The propose of this work was evaluated by the drug susceptibility *in vivo* and *in vitro* of *P. falciparum* to chloroquine (Cq) and to the sulfadoxine-pyrimethamine (Sp) combination, in Domingo Sifontes municipality between October 1994 and December 1995. Clinical-epidemiological study was performed in 70 patients (14-66 years old) and the 88.5% were male. The *in vivo* study was performed on 35 patients for each study drug in agreement with World Health Organization recommendation. In relation to the Cq responses, eighteen cases (51.4%) resulted sensible to the drug; ten cases (28%) were RI; five infections (14.2%) were RII and two cases (5.7%) were RIII. To the Sp combination 20% of the cases demonstrated resistance at variable levels (IR : 17% and RII: 2.9%). The *in vitro* study was carried out using the Rieckmann's microtechnique. For Cq *P. falciparum* isolates demonstrated a 11% of resistance with values of EC50 and EC99 of 1.71 to 24.57 µmol/L. To the Sp combination a 89% of the isolates showed sensibility up to 95.77% of schizonts maturity inhibition between 0.01 and 1.027 µmol/L. A direct correlation between the location and the geographical dispersion and the diverse degrees of resistance were found. The high populational migration without any control, the bad therapy pressure, the automedication and the subcurative doses were analyzed in relation with the selection of resistant parasite populations in areas of high malaria transmission.

PS - 13.19

P Shahi

(Abstract not received)

PS - 14.1

DETECTION OF *GIARDIA LAMBLIA* ANTIGENS IN FAECES : "LATEX BEAD AGGLUTINATION TEST (LAT)".

Mamta Singh[1], A K Agrawal, Pramod Nath[2] and B Malviya[1]
1. Central Drug Research Institute, Lucknow (India)
2. Head, Post Graduate Department of Pathology, King George's Medical College, Lucknow.

Giardiasis is one of the most common intestinal infections with worthwide distribution. It is responsible for intestinal malabsorption, diarrhoea and profound weight loss. Diagnosis of the infection is difficulat due to the non-specificity of symptoms and low sensitivity of the traional diagnostic techniques. The conventional antibody directied tests often give false positivity due toe persistence of serum antibody much after the clinical cure of giardial infection. Biologically inert polyvinyl to toluene latex particles of appropriate and uniform size, when mixed with gamma globulin, substitute sheep-cells in diagnosis of giardiasis.

Giardial antigens purified by Sephadex-G-100 and C-52 (ion exchange) column-chromatography or by electro-elution was employed to raised hyper-immune serum (HIS) in rabbits. HIS at the lowest dilution of 1:20, 480 gave a positive agglutination with detection sensitiviof antigen upto 2.18 µg/ml. LAT, on comparison to IHA and ELISA, was found superior due to its high sentivity, least variability, long shelf life and the rapidity of test. Data comprising various aspects of development of this test would be presented.

PS - 14.2

FREEZE DRIED DAT ANTIGEN FOR SERODIAGNOSIS OF VISCERAL LEISHMANIASIS - A PRELIMINARY REPORT

Suman Gupta, Suman Tiwari, Ashim Kumar Bagchi, G K Jain and J C Katiyar
Division of Parasitology and Pharmaceutics, Central Drug Research Institute, Lucknow 226 001 (U.P.), India

The laboratory diagnosis of visceral leishmaniasis (VL) presently relies on the demonstration of *Leishmania* amastigotes in bone marrow/spleen aspirates. The procedures are painful, cumbersome and fairly inadequate in early phase of infection.

Of the currently employed tests (IFAT, CIE and DAT), DAT is considered most appropriate and field oriented (the test standardised by us is being marketed as 'LEISHMAN TEST' and used for diagnosis and seroprevalence of kala-azar). The test is simple, economical, field-based technique with higher sensitivity and specificity. The major drawback of the DAT is the limited stability of the liquid antigen at 4°C. In fields where no cooling facilities are available, a stable antigenic formulation would significantly

enhance its practical utility. With this aim we have developed a freeze dried antigen (FD antigen) for the detection of VL using specific freeze drying solution. Initial studies carried out with different categories of antileishmanial sera depicted that FD antigen is comparable in reactivity to liquid DAT antigen and has the major advantage of (i) long shelf life (ii) easy packaging and transportation (ii) reproducibility in results over a long period of time due to production of a large batch of antigen (iv) storage of ambient temperature.

PS - 14.3

PREVALENCE OF FILARIASIS IN CHEST SYMPTOMATICS - A PILOT STUDY

P K Murthy, R P Ghosh, S Sarin, P K Mukherjee, A R Sircar and J C Katiyar
Division of Parasitology, Central Drug Research Institute, Departments of Tuberculosis and Medicine, K.G's Medical College, Lucknow-226 001, India

In filaria endemic areas a large number of cases present chest symptoms like cough, breathlessness, chest pain, hylar lymphadenopathy and other lymphadenopathy. Proper diagnosis of such cases is often missed and at times wrongly diagnosed as tuberculosis. In a pilot study the prevalence of filariasis was investigated in 85 patients with diverse chest symptoms admitted to the Kasturba Chest Hospital (K.G.'s medical college, Lucknow) and/or coming to outdoor patients department (OPD) of the medical college. Patients categorised on the basis of clinical, radiological and bacteriological findings were tested for filariasis by filarial skin test (FST) using *Brugia malayi* infective larval antigen and technique. Patients of group I (36) showing asthma type symptoms and abnormal chest radiographs were highly reactive to FST antigen and 7 of them (19.4%) had mf in their blood. In group II, 9 of the 13 (69.2%) with pleural effusion showed positive FST, 6 of them demonstrated mf either in pleural aspirate or in the blood. Amongst 36 pulmonary tuberculosis patients (group III), 23 (63.6%) responded to FST antigen and 2 of them (5.6%) were positive for mf. Thus, based on FST which detects prepatent, latent and established filariasis, all respiratory symptomatic patients with high absolute eosinophil count revealed filarial positivity suggesting tropical pulmonary eosinophilia (TPE) syndrome. The incidence of pleural effusion and mf positivity suggested that mf could be responsible for the disease. Further filarial positivity in pulmonary tuberculosis patients indicated that most probably these patients were carrying prepatent or subclinical filarial infection. Six patients from different groups showing FST positive and demonstrating mf in the peripheral blood responded favourably to DEC treatment confirming the symptoms related to filariasis. The study emphasises that FST may be included as one of the routine tests in chest clinics to screen patients particularly coming from filaria endemic areas.

PS - 14.4

B Sriram

(Abstract not received)

PS - 14.5

A K Bagchi
(Abstract not received)

PS - 14.6

S Tiwari
(Abstract not received)

PS - 14.7

ROLE OF ULTRASONOGRAPHY TECHNIQUE IN HUMAN BANCROFTIAN FILARIASIS

Amita Shukla Dave,[1] N Fatma,[1] R K Gupta[2] and R K Chatterjee[1]
1. Division of Parasitology, Central Drug Research Institute, Lucknow-226 001.
2. Department of Radiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow-226 001 (India).

Lymphatic filariasis is a vector borne parasitic disease affecting 120 million people in 76 nations worldwide. Little is still known about the parasite *in situ.* Recently, non-invasive, imaging technique ultrasonography (USG) has been used to localize the live adult worms of *Wuchereria bancrofti* in the human host. With USG, nests of highly motile, live adult worms of *W. bancrofti* are now readily demonstrable in the scrotal lymphatics. In the present study filarial patients from endemic village of Bakshi-ka-Talab (Lucknow, U.P.) were initially identified using conventional night blood smear technique. A total of eleven patients were identified of these seven were symptomatic and four were asymptomatic microfilaraemic males. All the eleven subjects gave their consent to participate in the ultrasonographic study for localization of live adult filarids. They were thoroughly examined beforehand and found to be apparently free of ailments except for filarial manifestations and/or microfilaraemia. Patients were then screened with a 7.5 MHz transducer in an Ultramark 4 plus ultrasound system. Out of these eleven male patients, six showed presence of live adult worms (aleatoric movements or filaria dance sign; FDS) in the spermatic cord and inguinal glands. In the remaining five patients anechoic hydrocele was observed but no adult worms were seen. The patients which showed live adult worms in their scrotal lymphatics were picked up for DEC treatment. Different dose schedules of DEC were given to the patients and follow up of the cases is being carried out. Initial results indicate ultrasonography to be an important tool to observe directly the live parasites *in situ* and to monitor macrofilaricidal drug efficacy in human subjects.

PS - 14.8

DETECTION OF *PLASMODIUM* AND *VIVAX* FROM WHOLE BLOOD BY POLYMERASE CHAIN REACTION AND MICROTITER PLATE HYBRIDIZATION

M Patnaik and A Khachatryan

The use of 18S rRNA, genus-specific primer pairs with spacies specific probes for the detction and speciation of different *Plasmodium* species by PCR has been reported. We used a modified procedure of a previously described PCR-EIA mrthod using oligonucleotide probes coated on to microtiter plates for hybridization and detection of *Plasmodium falciparum* and *vivax* DNA. The following strains were obtained, (*P. falciparum*) : Vietnam FCR1/FVO, Gambia FCR3/FMG, Honduras-1, FCC-2/Hainan, FCR-3/Gambia subline F-86, FCR-3/Gambia clone D-3 knobless, FCR-3/Gambia clone D-4 knobless, FCR-3/Gambia clone I knobby, Clone A-2 knobby, HB-2 and HB-3; (*P. Vivax)*: NIH strains Chesson, W. Pakistan, NICA Paname, S. Vietnam II, S. Vietnam, Sal I, Sal II and Cambodian.Parasitemia livels ranged from 6.7% to 0.1%. Whole blood was lysed using saponin and RBC were removed by centrifugation at 3000 rpm. The pellets were then incubated at 95 degrees for 10 minutes after which PCR amplification was performed in a GeneAmp 9600 thermocycler. Denaturation solution was added to all the samples and hybridization performed on microtiter plates previously coated with *vivax* and *falciparum* - specific oligonucleotide probes, Avidin-HRP conjugate (1 mg/ml) and TMR substract solution is used to develop the color reaction which is stopped after 10 minutes with 5% sulfuric acid. All of the above mentioned strains of *P. falciparum* and *P.vivax* were detected using this protocol. Samples were diluted with uninfected whole blood to assay different parasitemia levels. We detected all the samples by both ethidium bromide stained agarose gel electrophoresis as well as microtiter plate hybridization. The detection limited of the microtiter plate method was 0,00005% (2 parasites/µl) which was two log levels greater than that detected by the gels. No cross reactivity was observed with other species of *Plasmodium* or other parasitic, bacterial or viral deseases. Both drug resistant and susceptible strains are detected by this method which has the advantage of being highly sensitive and specific and relatively incxpensive, further large scale studies are underway to use this test in field studies and vaccine trials. A quantitative method is being developed to be used in testing for drug resistance in malaria.

PS - 14.9

CLINICAL EVALUATION AND SURVEY OF CAUSATIVE ORGANISMS IN INFANTS AND YOUNG CHILDREN'S DIARRHOEA IN RURAL AREAS OF LUCKNOW

M Divya Jyothi[2] and N Bora[1]

1. Department of Pathology, Sewa Hospital and Research Centre, Lucknow.
2. Division of Microbiology, Central Drug Research Institure, Lucknow-226 001

Diarrhoea and gastroenteritis (diarrhoea with vomiting) are major causes of death and ill health in many developing countries especially in India. Clinical and laboratory studies on total of 250 infants and young children admitted to the hospital for various types of diarrhoea during 1994-1995 are described. Different type of preparations, staining procedures, cultural methods and biochemical tests have been performed to find out the causative organisms. The major bacterial pathogen was enteropathogenic *Escherichia coli* (20.8%). Others were *Shigella* sps (10.1%), *Salmonella* sps (6.8%), *Vibrio cholera* (2.8%) and *Lactobacillus* (2%). Most of them were resistant to Tetracyclin, Gentamycin, Ampicillin, Bacitracin and Chloramphenicol & sensitive to Nalidixic acid, Co-trimoxazole, Norfloxacin and mildly to Erythromycin, other major enteropathogens were *Entamoeba histolytica* (15.2%), *Giardia lamblia* (12%), *Campylobacter jejuni* (7.2%), *Clostridium perfringens* (0.8%). *Ascaris lumbricoides* (8%) and *Ancylostoma duodenales* (6.8%) were also found. Some of the cases were found to be due to lactose deficiency (7.5%). Many of the patients had complications with marked dehydration, hypoelectrolytaemia, malnutrition and infections.

The study showed that diarrhoea incidence peaked in the age group around one year, coinciding with the time of greater exposure to enviromental contaminats. Timely rapid rehydration and restoration of electrolytes followed by suitable management of complications are necessary.

PS - 14.10

IN VITRO CLINICAL CORRELATE FOR ASSESSMENT OF PATIENTS' RESPONSES TO CHLOROQUINE AGAINST *PLASMODIUM FALCIPARUM* INFECTION.

Sukla Biswas, Neena Valecha, M A Ansari and V P Sharma
Malaria Research Centre, 22, Sham Nath Marg, Delhi - 110054 (India)

Chloroquine sensitivity was tested in a group of patients with microscopic positivity for *Plasmodium falciparum* from five different regions of the country using *in vitro* and *in vivo* tests. Patients were treated with chloroquine at the total dose of 25 mg/kg body weight for three consecutive days. Then they were followed up for a period of 28 days using standard test method. Recrudescence was observed in 29 patients (29.6%) out of 98 while others responded to the drug as no parasitaemia was observed in peripheral smears on day 7-10 and on day 28. *In vitro* drug sensitivity test was carried out in all 98 samples prior to treatment. Results demonstrated that in 69 positive responders, the average minimum inhibitory concentration fell below 1.14×10^{-6} M and in 29 recrudescent cases the inhibition of growth or maturation was observed at above 2×10^{-6} M. *In vitro* microtest technique was applied in a village based survey and a positive correlation was observed among drug responders and susceptibility of parasites to various doses of chloroquine.

PS - 15 .1

DYNAMICS OF MALARIA TRANSMISSION IN THE FOREST AREA OF FOOTHILL REGION OF LESSER HIMALAYA, INDIA

R P Shukla[2], S N Sharma[2], V K Kohli[2] and V P Sharma[1]
1. Malaria Research Centre, 22, Sham Nath Marg, Delhi - 110054
2. Malaria Research Centre, Field Station, Bhotia Parao, Inderjeet Garden, Haldwani, Distt. Nainital Pin - 263 141

In eighties high malaria incidence and deaths occured in Terai regions. But after mid nineties high malaria cases were reported in the forest area of the foothills of Nainital District. Therefore, to determine dynamics of malaria transmission in the forest region of Nainital, entomological and parasitological investigations were carried out in two villages viz. Haspur and Jolasal from July, 1996 to March, 1997.

Entomological investigations included extensive and intensive larval surveys, man hour densities, resting and biting behaviour and vector incrimination. Both *A. culicifacies* and *A. fluviatillis* were found breeding in the rivulets and streams of the area. *A. culicifacies* densities varied from 1.0 to 201.0 with a peak in the month of Augus, whereas, *A. fluviatilis* densities varied from 8.0 to 115.0 with highest density recorded in the month of December. High densities were observed in cattle sheds as compared to human dwellings. Results on man vector contact revealed high biting activity in outdoor as compared to indoor during July to September.

823 and 107 specimens of *A. fluviatilis* and *A. culicifacies* were dissected for natural infection of sporozoites in salivary glands. Only one (0.12 spz rate) specimen of *A. fluviatilis* was found harbouring sporozites int hemonth of Nov., 1996. No infection was detected in *A. culicifacies*.

Active fever survey revealed spr 54.7 and Sfr 42.6. Overall results revealed active role of *A. fluviatilis* in Malaria transmission. However, *A. culicifacies* densities were also recorded high in the area.

PS - 15.2

LABORATORY TRIALS OF PREDATORY INSECTS AGAINST ANOPHELINE LARVAE

M S Malhotra
Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.

Local insects, predatory to anopheline larvae were collected from dams, ponds and rivers of Sitarganj, Bazpur, Kashipur, Lamachaur and Khatema of district Nainital. Insects were settled in laboratory in different aquaria. The counted number of *Anopheles* larvae alongwith *Daphnia* sp. and *Cyclops* were released in experimental aquaria.

Anopheline larvae were visibly counted at one hour interval. The experiments were replicated several times and study lasted over one year. Study revealed that adult *Cybister* species (Coleoptera) consumed on an average over 25 larvae in 2 hours. Next to follow were back swimmers *Enitheres indica* and *Anisops* sp. which killed and sucked about 20 larvae during first two hours almost consistently. *Cybister* sp. larvae were found to be voracious eaters of anopheline larvae (50/24 hrs.), but they also consumed *Daphnia* sp.

Gerris and dragon fly larvae were poor predators destroying less than 5 larvae in 24 hrs. Studies also revealed that *Hydrometra* sp. and *Nepa* sp. were not able to destroy anophelines and thus proved to be unsuitable for mosquito control.

Daphnia and *Cyclops* were introduced in aquaria to observe the food preferences, if any, of the predatory insects.

PS - 15.3

SEASONAL PREVALENCE OF ANOPHELINES IN SHAHJAHANPUR DISTRICT, UTTAR PRADESH.

S N Sharma and R P Shukla
Malaria Research Centre, Field Station, Inderjeet Garden, Bhotia Parao, Haldwani- 263 141 District Nainital.

Information on anopheline prevalence particularly the vector species is required for proper planning of malaria control activities. Practically very little has been published regarding the seasonal prevalence of different anophelines in Shahjahanpur district where malaria epidemics had occurred in the past. Longitudinal surveys from Feb. 1987 to Dec. 1991 were carried out for collection of adult mosquitoes from 2 villages of Shahjahanpur. Results revealed prevalence of 10 anophelines in the area. Specieswise percentage composition was. *An. annularis* 39.2%. *A n. subpictus* 31.5%, *An. aconitus* 15.0% *An. culicifacies* 12.9%, *An. pallidus* 0.5%, *An. splendidus* 0.5%, *An. barbirostris* 0.2% , *An. nigerrimus* 0.2%, *An. stephensi* 0.1% and *An. vagus* 0.02%, An. anophelines, *viz; An. culicifacies, An. annularis, An. subpictus* and *An. aconitus* were predominantly prevalent. The other above mentioned 6 species were found in low number. Both *An. culicifacies* and *An. annularis* prevailed all through the year with densities ranging from 0.8 to 37.2 and 1.0 to 106.0 respectively. High density of *An. culicifacies* was found during July to Sept. and low during Jan and Feb., *An. annularis* prevailed with higher densities during Oct. to April. The prevalence pattern of *An. aconitus* was similar to that of *An. annularis*.

High densities of *An. subpictus* occurred during July to Sept. and low during Jan. to April. *An. splendidus, An. pallidus, An. nigerrimus* and *An. barbirostris* prevailed during post-monsoon and winter months. Both *An. stephensi* and *An.vagus* showed erratic

prevalence. Overall results of the 5 year study revealed marked difference in the seasonal prevalence of different species.

PS - 15.4

SEASONAL PREVALENCE OF ANOPHELINES WITH SPECIAL REFERENCE TO BIONOMICS OF VECTORS OF MALARIA IN ASSAM

Vas Dev
Malaria Research Centre (ICMR), Field Station, Sonapur, Assam, India.

Year round collections of anopheline mosquitoes from cattle sheds, human bait and indoor day resting populations revealed the presence of 23 species in the Brahmaputra valley of Assam. Of these, *An. philippinensis* was the most prevalent while *An. vagus* and *An. hyrcanus* group comprised the fair proportion of the fauna. Majority of the species were encountered in the cattle sheds after dusk, and were most abundant during May to October (wet season). In the day resting catches from human dwellings (indoor), *An. minimus* comprised the most and were prevalent throughout the year, the maximum abundance occurred during March till August. In the over night human bait catches as well, *An. minimus* was the most abundant, and fed all through the night. Its peak biting activity occurred during 01:00 to 04:00 and the biting rate ranged between 1.00 to 15.83 per man per night, the highest being during May and June. Of various species dissected for sporozoites, *An. minimus* were incriminated, and sporozoite infections were recorded practically for all 12 months; the highest infection rate (8.51) was recorded in October. Sporozoite infections were also recorded in *An. fluviatilis* but its prevalence was very low and seasonal (winter months). *An. dirus* (the other potential vector) were recorded during monsoon months but in low numbers and could not be incriminated. All these species were found breeding in wide variety of habitats but *An. minimus* was species specific ; its breeding occurred all through the year in slow flowing perennial streams with grassy margins.

PS - 15.5

M H Hodjati
(Abstract not received)

PS - 15.6

R S Pandian
(Abstract not received)

PS - 15.7

LIFE TABLE ATTRIBUTES OF *ANOPHELES CULICIFACIES* SIBLING SPECIES

T S Sathyanarayan[1], S K Subbarao[2], T R Rao[3], and V P Sharma[2]
1. Malaria Research Centre, Bangalore.
2. Malaria Research Centre, Delhi.
3. Department of Zoology, University of Delhi, Delhi.

Experimental studies were carried out under laboratory conditions for estimating the life table attributes (both for larval and adult stages) of sibling species of *An. culicifacies* complex. From the survival rates of immature stages it was seen that species A had higher larval mortality rates, longer pupation and emergence times than other two species. Horizontal life table experiments showed that (1) the adult survivorship patterns were similar for female mosquitoes in all the three sibling species, but species B had higher longevity than others, (2) the longevity of males was shortest in species B and (3) the gross and net reproductive rates as well as the intrinsic rates of natural increase were highest in species B and lowest in species A. Species C had a significantly longer generation time than species A and B. Two epidemiologically important parameters, the expected life of infectivity and the probability of survival through sporogonic cycle were calculated for species A based on field data. The daily survival rate of species A was 0.834 under field conditions. The probability of survival through sporogonic cycle and the expected life of infectivity for species A were 0.195 and 3.8 for *P.vivax* and 0.163 and 3.6 for *P. falciparum* respectively. The durations of gonotrophic cycles I and II were 4 and 2 days respectively. Epidemiologically, species B unlike species A has rarely been incriminated in malaria transmission in India. The present study clearly shows that none of its life history parameters such as female longevity, fall short of the requirements to be an effective vector, relative to species A (which is a major vector). Greater propensity for zoophagy of species B might be one of the factors and longevity of this species has to be determined in the field conditions

PS - 15.8

MORPHOLOGICAL AND HISTOPATHOLOGICAL EFFECTS OF A CHITIN SYNTHESIS INHIBITOR ON MOSQUITOES

R Mohapatra M R Ranjit and A P Dash
Regional Medical Research Centre, Bhubaneswar - 751 016 Orissa, India.

Hexaflumuron [N - (((3, 5 - dichloro - 4 - (1, 1, 2, 2 - tetrafluoroethoxy) phenyl) amino) carbonyl) - 2,6 difluorobenzamide] a chitin synthesis inhibitor caused a range of delayed morphogenetic aberrations in different developmental stages of mosquitoes when treated at the late third instar larval stage. The most common effects at lower concentrations were formation of swollen headed curved larvae (34 - 35%), larvae with black abdomen (12%), albino larvae (2-5%) and adultoid pupae (1-2%) in different

mosquito species *viz., Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus.* Hexaflumuron also induced atrophy of striated thoracic and abdominal muscles, karyorrehxis of the malpighian tubules and atrophy of ovary. Besides this it was also observed to inhibit the formation of cuticular system.

PS - 15.9

T Vij
(Abstract not received)

PS - 15.10

INHERITANCE AND LINKAGE OF *BACILLUS SPHAERICUS* RESISTANCE IN *ANOPHELES STEPHENSI*

PK Mittal, T Adak and V P Sharma
Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.

Bacillus sphaericus and *Bacillus thuringiensis* H-14 are two microbial agents that have been evaluated extensively as bio-insecticide to control vectors of malaria and other diseases. After one year of application of *Bacillus sphaericus* in the field to control mosquito breeding, resistance was detected in *Cx. quinquefasciatus.* Subsequently, resistance to *B.sphaericus* toxins in a naturally tolerant strain of *An. stephensi* was detected and this strain is being maintained in the laboratory.

We report here the inheritance and linkage of *Bacillus sphaericus* resistance in *An. stephensi.* For this purpose, resistant strain (Lc50>1600 mg/1) was crossed reciprocally to a susceptible strain *golden yellow larva* (Lc50=0.08 mg/1). The F_1 progenies of both the reciprocal crosses were inbred and backcrossed. Results of these crosses revealed that resistance is recessive and autosomally inherited. No linkage between *B.sphaericus* resistance and *golden yellow larva* was established. As *golden yellow larva* is on chromosome 3, *B. sphaericus* resistant gene has been assigned to linkage group II. Implications of these finding would be discussed in the background of available vector control tools.

PS - 15.11

MALARIOGENIC POTENTIAL OF NATIONAL THERMAL POWER CORPORATION (NTPC) SHAKTINAGAR, DISTRICT SONBHADRA (U.P.)

Virendra K Dua[1] and V P Sharma[2]
1. Malaria Research Centre, Field Station, BHEL, Ranipur, Hardwar 249 403
2. Malaria Research Centre, 22 Sham Nath Marg, Delhi-110 054 India.

NTPC Shaktinagar was surveyed for malariogenic conditions from 1994 to 1996. The major mosquito breeding sites were i) drains, ii) storm water drains, iii) lake, iv) outside

tanks, v) over head tanks, vi) sluice valve chambers vii) ornamental tanks, viii) wells, ix) pit wells and x) water reservoir etc. *A. culicifacies* was found major vector of malaria in this area. Sibling species identification of *A.culcifacies* revealed that species C dominated during the months of Sept. to Nov. every year. The insectticide susceptibility tests against *A. culicifacies* showed that *A.culicifacies* population was sensitive of malathion, deltamethrin and fenitrothion while it was found 44% resistant of DDT. The malaria cases recorded in 1994, 1995 and 1996 were 847, 590 and 409 respectively. *In vitro* study on *P. falciparum* cases showed that 40, 70 and 50% isolates were resistant to chloroquine in 1994, 1995 and 1996 respectively while in vivo follow-up resulted only 20-30% *P. falciparum* cases resistant to chloroquine. All chloroquine resistant cases responded well to Fansidar treatment. Our surveys revealed that malaria cases decreased due to effective intervention measures, prompt cases detection and proper treatment as compared to previous years.

PS - 15.12

STUDIES ON HEAT SHOCK PROTEINS IN *AN. STEPHENSI* LARVAE

K Raghavendra, S K Subbarao, T Adak and V P Sharma
Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054.

All organisms respond to heat by inducing synthesis of a group of proteins called heat shock proteins or *HSPs*. These proteins are the most highly conserved proteins in existence. Larvae from malathion-resistant and - susceptible *An. stephensi* lines were exposed to different temperatures viz., 37°C, 39°C, 41°C, 43°C and 45°C. Complete survival of larvae and emergence to adults were observed at 37°C and 39°C while from 41°C onwards increasing mortalities were recorded and at 45°C the larvae died within 5 min of the exposure. Increased thermal tolerance at higher temperatures was observed in larvae which were preadapted at sublethal temperatures. Variation in insecticide susceptibilities was also observed when exposed to insecticides. Limited experiments to study the susceptibility to malarial parasite did not show significant difference between preadapted and non-adapted larvae ($\chi^2 = 0.765$). Detailed results of the above studies will be presented.

PS - 15.13

FACTORS AFFECTING MOSQUITO LARVAL ABUNDANCE IN RICE AGRO-ECOSYSTEM OF KHEDA DISTRICT OF CENTRAL GUJRAT

Rajani Kant[1], S D Pandey[2] and V P Sharma[3]
1. Present address : ICMR, Ansari Nagar, New Delhi-110 029.
2. Christ Church College, Kanpur-208 001.
3. Malaria Research Centre, 20 Madhuban, Delhi-110 092.

Ecological studies were conducted on mosquito breeding pattern and factors affecting immature larval population in an irrigated rice agro-ecosystem of Kheda district of Central

Gujarat. A total of 30 mosquito species representing six genera viz. *Anopheles, Culex, Aedes, Mansonia, Ficalbia* and *Mimomiya* were encountered inhabiting this water ecosystem. *An. subpictus, An. culicifacies, An. annularis, An. nigerrimus* and *An. tessellatus* constituted the bulk of anophelines, whereas, *Cx. vishnui* gr. dominated the culcine composition. Breeding of *An. subpictus* and *An. culicifacies* was found inversely proportional to the height of plants which declined with the increase in plant height, canopy development and reduced interspace in later stages of the crop and was replaced by more shade loving species such as *An. nigerrimus, An. barbirostris, An. tessellatus* as algae, *Lemna, Hydrilla* and *Ipomea* also supported some of the species whereas, application of agrochemicals reduced the larval density during the period of its application. Field distance from human habitation, rice variety, water depth and cultural practices were also found to affect the breeding and prevalence of mosquitoes. Interspecific associations between species also resulted due to presence of similar micro-climatic conditions however, negative associations were due to interspecific repulsion or change in the breeding behaviour of two species.

PS - 15.14

STUDIES ON THE BIONOMICS OF MALARIA VECTOR *AN. FLUVIATILIS* JAMES IN THE FOREST AND BROKEN FOREST AREA OF SUNDARGARH DISTRICT (ORISSA)

S K Sharma, A K Upadhyay and V P Sharma[1]
Malaria Research Centre, Field Station, Civil Township, Rourkela-769 004, Orissa, India.
1. Malaria Research Centre, 22- Sham Nath Marg, Delhi-110 054.

An. fluviatilis James belonging to the series Myzomia of the subgenus *Cellia* is among the most efficient vectors of malaria particularly in hills and foothills of India. It is widely distributed in India although its role in malaria transmission varies from place to place. Recent study has shown that *An. fluviatilis* is a complex of three reproductively isolated populations that can be cytologically identified. Since these species do nto interbreed in nature they are expected to show biological variations. Keeping in view these facts and also varied ecological conditions prevailing in the tribal district of Sundargarh (Orissa) characterized by persistant malaria transmission, a study on the bionomics of *An. fluviatilis* in two distinct ecological conditions i.e forest and broken forest area was initiated. Larval surveys indicated that the species prefer to breed in the slow running streams which are much more abundant in the forest area as compared to the broken forest area. Results of the monitoring of the adult density of the species indicated that April to August were the months of low prevalence and the density started building up soon after the monsoon rains and reached peak in the month of January and sufficient population was maintained up to March in the forested area whereas the density decrease was very sharp after January in the broken forest area. Whole night biting collections revealed that the species prefer to bite on human beings sleeping outdoor. A biting rate of 1.7 and 3.5 bites/man/night was observed on indoor and outdoor man

biting collections respectively in the forest area whereas a very low of 0.3 and 0.8 was observed on indoor and outdoor biting collections respectively in the broken forest area. Human biting continued throughout the night starting between 1900-2000 hours and ending in the early part of the morning (0400-0500 hours). Maximum biting was observed between 2200-2300 hours. The cytological examination of the species revealed that species S is predominant in the area which is characterised by low densities but very high anthropophilic index, thereby playing major role in transmission of malaria in the forest area whereas it plays complimentary role with *An. culicifacies* in malaria transmission in the broken forest area.

PS - 15.15

T Adak
(Abstract not received)

PS - 15.16

K Narang
(Abstract not received)

PS - 15.17

EVALUATION OF INSECTICIDE - IMPREGNATED BED NETS AGAINST *ANOPHELES SUNDAICUS* IN ANDAMAN AND NICOBAR ISLANDS, INDIA.

M K Das[1], T Adak[2], and V P Sharma[2]
1. Malaria Research Centre, F S Malacca, Car Nicobar Island-744 301, A & N Islands. India
2. Malaria Research Centre, 22 - Sham Nath Marg, Delhi-110 054, India

The insecticide (Cyfluthrin) impregnated bednet (IIBN) trial in the defence area Campbell Bay, A & N Islands has been started. Baseline entomological data were collected. Seven night catches (dusk to dawn) and morning collections were carried out before the commencement of the trial and also after using impregnated bednets. Before the trial started, the mean number of *Anopheles sundaicus* bites was 37.7 bites/man/night while after the introduction of the cyfluthrin impregnated mosquito nets *Anopheles sundaicus* biting on human baits inside the partially lifted nets was reduced to zero.

PS - 15.18

ISOENZYME ANALYSIS OF *ANOPHELES CULICIFACIES* COMPLEX

Suman Wattal[1], T Adak[1], V P Sharma[1] and A R Khuda-Buksh[2]
1. Malaria Research Centre, 22-Sham Nath Marg, Delhi-110 054, India.
2. Kalyani University, Kalyani (W.B), India.

A study of isoenzyme variability was undertaken with the objective of measuring the genetic variability and finding diagnostic enzymes to distinguish different members of *Anopheles culicifacies* complex. Electrophoretic data were analysed using the BIOSYS-I Computer program. Allelic frequencies, genetic variability, Hardy-Weinberg equilibrium, fixation index, F-statistics, and Nei's genetic distance and similarity coefficients in pairwise comparisons between different sibling species were estimated. Results indicated very close genetic similarity and low genetic diversity between species B and species C as compared to other pairs. Detailed results and their implications in disease transmission will be discussed.

PS - 15.19

CONTROL OF *AEDES AEGYPTI* BREEDING

C P Batra, P K Mittal and T Adak

In recent years the epidemics of dengue fever and dengue haemorrhagic fever (DHF) in many parts of India is a major concern among the public health experts. The population of *Aedes aegypti* which transmit DHF has increased unprecedently due to rapid urbanization, industrialization, water storage practices, piped water supply in rural areas and population migration. This mosquito breeds mainly in containers, desert coolers, unused tyre dumps where application of any vector control method is very difficult.

Three different formulations *viz.* neem water emulsion, microbial agent *Bacillus thuringiensis* (both water disposable powder and granules) and Baytex granules were tested in the control of mosquito breeding.

Application of 10% neem oil water emulsion @40 to 80 ml / breeding habitat resulted in complete inhibition of *Ae. aegypti* pupal production upto 14 days.

Two *Bacillus thuringiensis* formulations Bacticide and Vectobac granule were filled in gelatin capsules @1 gm and 2gm respectively per breeding structure showed 100% control for two weeks. Treated breeding habitat with 2% Baytex granules prevents *Ae. aegypti* breeding upto 5 weeks period.

Advantages of using these formulations with an innovating cost effective application technology over conventional methods will be discussed.

PS - 15.20

PREVALENCY OF LIVER FLUKE METACERCARIAE IN DIFFERENT LARVAL STAGES OF *ANOPHELES STEPHENSI*

B P Pokhriyal, R K Mahesh, C P Singh and R K Jauhari
Parasitology Laboratory, Department of Zoology, DAV (Post Graduate) College, DehraDun - 248 001, India.

Very little compendium of references is available regarding the deployment of trematode parasites such as cercariae and metacercariae for the control of mosquitoes (Rao et al. 1985, Dempster et al. 1986 and Dempster and Rau, 1990). Biswas et al. (1987) found an association between mosquito larvae and scavanger snails where the growth of larvae, onset of population, duration of pupal stage and emergence of adult mosquito was efected by the secretion of the scavanger snail *Lymnaea sp.*

The present investigation is an attempt to study deployment of metacercariae in the larvae of *An. stephensi* at Laboratory conditions.

The present investigation reveals laboratory study on the adaptation of liver fluke cercariae to different stages of *Anopheles stephensi* larvae. The prevalence of liverfluke metacercariae has been found more in IVth instar larvae than other stages. With regard to distribution of metacercariae they insisted more in the abdomen in comparison to thorax and the head region. The encystment had been found comparatively higher in IVth instar than IIIrd and IInd instars.

PS - 15.21

STUDIES OF BIOCONCENTRATION OF ORGANOCHLORINE INSECTICIDE RESIDUES IN AQUATIC STAGES OF *ANOPHELES CULICIFACIES* IN INDIA

Roop Kumari[1], V K Dua[2] and V P Sharma[2]
1. National Malaria Eradication Programme, 22-Sham Nath Marg, Delhi-110 054
2. Malaria Research Centre, 22-Sham Nath Marg, Delhi - 110 054

Extensive use of insecticides has led to development of resistance in disease vectors. *An. culicifacies*, a major vector in India has become resistant to DDT and HCH at most of the places. Present study was conducted to determine the level of organochlorine residues in mosquito larvae in order to established the effect of the insecticides on aquatic stages of mosquito and in development of resistance mechanism. Larvae and water samples were collected from different breeding habitats of *An. culicifacies* mosquito during the month of August to October, 1994 from Haridwar and Rishikesh and analysed for HCH and DDT residues. Results indicate that in water samples collected from different breeding habitats, the mean level of HCH was 6.702 µg/L and that of DDT was 4.837µg/L. Mean HCH concentration of external extract of the larvae was 8.381 mg/kg, while mean value of internal HCH contents was 12.734 mg/kg. Similarly, DDT residues in external and internal extract of *An. culicifacies* larvae was 5.332 mg/kg, and 8.805 mg/kg, respectively. The bio-magnification factor (from water to internal larvae) was found to be 1961 for HCH and 1831 for DDT. A highly significant correlation was observed for HCH and DDT residues between water and internal extract ($r=0.938; P<0.001$ and $r=0.904; P<0.001$, respectively), between water and external extract ($r=0.917; P<0.001$ and $r=0.923; P<0.001$, respectively), and between internal and external extract of larvae ($r=0.977; P<0.001$ and $r=0.923; P<0.001$, respectively). Present

study shows that when larvae come in direct contact with insecticide residues in contaminated water of breeding habitats, they absorb large amount of insecticides residues, metabolise and may develop resistance while in aquatic stage only without even coming in direct contact with residual spray in adult form. This also explain the development of resistance of mosquito vector at places which have not seen pesticides spray under public health programme in past.

PS - 15.22

K R Kumari
(Abstract not received)

PS - 15.23

OBSERVATION ON THE SURVIVAL AND DEVELOPMENT OF *ANOPHELES CULICIFACIES* (DIPTERA : CULICIDAE) LARVAE IN THE RICE FIELDS OF DEHRADUN VALLEY

R K Mahesh and R K Jauhari
Parasitology Laboratory, Dept. of Zoology, DAV (PG) College, DehraDun (UP), India.

Rice fields have attracted increasing attention as breeding sources of vector mosquitoes, particularly in developing countries where rice land area is rapidly expanding to meet the urgent need for food. It seems essential to plan strategies aimed at exploiting naturally breeding mosquitoes by nature predators as the cost of insecticides is rising on one hand and its adverse effects to the environment on the other hand.

The present study reveals survival and development of Anopheles culicifaceis larvae under natural conditions in the rice fields in DehraDun Valley. The predators recorded in the experimental rice fields were insects belonging to Odonata, Hemiptera and Coleoptera while fishes belonged to Abramaininae and Cyprininae. The larval emergence rate was found higher in absence of nature predators. Water level and surface area of the rice fields also influenced the servival of larvae.

PS - 15.24

ECOLOGICAL ASPECTS OF TREMATODE INFECTIONS IN SOME COMMERCIALLY IMPORTANT CARANGIDS OF VISAKHAPATNAM COAST

U Sai Jyoti, and G Gnana Mani
Department of Zoology, Andhra University, Visakhapatnam - 530 003.

An investigation on trematode infections of commercially important carangids of Visakhapatnam coast, Bay of Bengal was undertaken during August, 1994 to July, 1996. Altogether, 740 fish belonging to five species namely *Carangoides oblongus, C. malabaricus, C. ferdau, Caranx ignobilis* and *C. carangus* were examined as monthly

samples. Data on host factors such as size, sex, feeding habits and reproductive status were recorded. Also data on species of parasites encountered, their abundance and location in the host were recorded. The parasite species found were identified and details of morphology and host specificity were investigated. From the data collected, the prevalence and mean intensity of infection with each parasite species in relation to host demographic factors and season were determined. Altogether, 13 species of trematodes including 4 species of monogeneans and 9 species of digeneans spread over 9 families were found. *C. malabaricus* and *C. ferdau* were found to be suitable hosts for trematode infections each harbouring 9 species of trematodes. An increase in the overall prevalence of infection with increasing host size was observed in *C. malabaricus* and *C.ignobilis*. The monogeneans were found to be host specialists and the digeneans were generalists in nature. It is concluded that the trematode fauna is rich and diverse and is a reflection of the host's varied diet.

PS - 16

COMPOSITIONAL HETEROGENEITY IN MOSQUITO GENOME

G Venkatachalaiah and M C Pandiyan
Centre for Applied Genetics, Department of Zoology, Bangalore University, Bangalore - 560 056, INDIA.

Among mosquitoes, anophelines show heteromorphic X-Y pair while most culicines (for eg., *Aedes, Culex* and *Mansonia*) sex chromosomes are not readily distinguishable. An attempt was made to throw some light on some aspects of basic chromosomal architecture to delineate whether compositional difference exists between male and female cells and of Anopheline and Culicine genomes.

Applications of classical C-staining studies on the genome have revealed that extensive constitutive heterochromatin was found over the paracentromeric regions of autosomes and most of sex chromosomes in anophelines, whereas in culicines staining profiles are confined to centromeric regions. An attempt was made to procure data on chromosome morphology in response to *in vivo* effect on some DNA-specific ligands (BrdU, Hoechst 33258, Ethidium Bromide, Acridine Orange and 5-aza cytidine) on the embryonic and adult chromosomes of *Anopheles* (*An. stephensi* and *An. nigerrimus*), *Culex* and *Aedes* and of subsequent staining by appropriate protocols. The present work deals with experiments to demonstrate that the pretreatment of live cells to various DNA-ligands have altered the basic architecture of chromosomes, in particular to heterochromatin compaction.

PS - 17.1

IN VITRO BIOLOGY OF A PIGEON WING LOUSE, *COLUMBICOLA COLUMBAE* LINN. (PHTHIRAPTERA : ISCHNOCERA)

S K Singh[1], Surman A Kumar[2] and A K Saxena[1]
1. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Kotdwara (Pauri Garhwal), India.
2. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Rishikesh (Dehradun), India.

Experimentations on *in vitro* rearing of Mallophage (Sens. Lat. Phthraptera) have always remained a challenging task for the Phthirapterologists. A few workers have made half-hearted attempts to demonstrate the *in vitro* biology of selected ischnocerans, from time to time. Present paper furnishes detailed information on *in vitro* biology of the pigeon wing louse, *Columbicola columbae*. Firstly, colonies of lice were reared at different temperatures (32-38°C), R.H. (70-90%) on feather diet to determine optimum conditions for survival and reproduction. Then, pairs of fresh looking adults were raised in separate vials till death to determine the adult lifespan, preoviposition period and total egg production by female during life time. Furthermore, freshly laid eggs were reared till final moult to record the incubation period and the duration of different nymphal instars. The results and methodology have been discussed in the paper.

PS - 17.2

EFFECT OF DIFFERENT TEMPERATURES AND RELATIVE HUMIDITY ON THE DEVELOPMENT AND HATCHING OF EGGS OF POULTRY SHAFT LOUSE, *MENOPON GALLINAE* LINNE. (PHTHIRAPTERA : AMBLYCERA)

Surman[1], S K Singh[1], A Kumar[2] and A K Saxena[1]
1. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Kotdwara (Pauri Garhwal), India.
2. Parasitology Laboratory, Deptt. of Zoology, Govt. P.G. College, Rishikesh (Dehradun), India.

Eggs of poultry shaft louse, *Menopon galliane* were reared at different temperatures and R.H. to determine optimum environmental conditions for development and hatching. Percentage hatch at 20, 25, 30, 35, 40 and 45°C. and 75% R.H. remained nil, 4.16, 19.35, 78, 82.85 and nil respectively. The incubation period has been found to be 8.0, 7.3 ± 1.36, 5.18 ± 0.8 and 4.79 ± 0.72 days at 25, 30, 35 and 40°C. respectively.

In another set of experiments, fresh eggs of *M. gallinae* were reared at different R.H., at 35 ± 1°C. Percentage hatch remained 62.5, 69.04, 78 and 61.5% at 33, 50, 75 and 90% R.H. Likewise incubation period has been recorded as 5.2 ± 1.08, 4. 89 ± 0.82, 5.18 ± 0.8 and 5.33 ±1.3 days, at the above said R.H.

Thus, it has been found that lower temperature causes reduction in percentage hatch and incubation period becomes prolonged. However, R.H. does not seem to have greater influence on percentage hatch and incubation period of the louse.

PS - 17.3

COMPARATIVE SUSCEPTIBILITY OF *PLASMODIUM KNOWLESI* AND *PLASMODIUM FALCIPARUM* TO ANTIMALARIAL DRUGS *IN VITRO*

Ragini Srivastava, S D Srinivas and S K Puri
Division of Microbiology, Central Drug Research Institute, Lucknow - 226 001.

Long term *in vitro* cultivation of most important human malaria parasite *P.falciparum* has greatly facilitated studies on basic biology and monitoring of drug response. The *in vitro* cultured parasites have been extensively used for evaluation of novel chemotherapeutic agents since *in vitro* cultures improve the ease of manipulation of large number of factors likely to influence the response. Non-availability of quality human serum which remains an essential prerequisite for successful cultivation, has hindered a wider use of this parasite in the developing countries. We report successful *in vitro* cultivation of a simian parasite *P. knowlesi* and argue for its application as a model of choice for chemotherapeutic studies. *Plasmodium knowlesi* produces synchronous infection in rhesus monkeys with quotidian periodicity. Short term cultures initiated with ring stage infected cells in 96 well microtitre plates have been successfully used to obtain dose response curves for reference antimalarials. The culture medium consisted of RPMI-1640 supplemented with 25 mm HEPES, 1% glucose, 0.23% $NaHCO_3$ and 10% monkey serum. The plates were incubated at 37°C in a candle jar. The relative maturation into schizonts at different concentrations was determined by microscopic observation of Giemsa stained blood smears and analysed to calculate the inhibitory concentration for each drug. The response of the drugs was similarly monitored against Indian isolates of *P.falciparum* (FCD-3 and FID-3) maintained in long term cultures using 10% human serum. The inhibitory concentrations for standard antimalarials have been compared the simian and human parasites. The advantages of *P.knowlesi* as an *in vitro* model for drug assay will be discussed.

PS - 17.4

LONG TERM CULTIVATION OF *PLASMODIUM KNOWLESI* AND ITS APPLICATION TO CHEMOTHERAPEUTIC STUDIES

S.D. Srinivas, Ragini Srivastava and S K Puri
Division of Microbiology, Central Drug Research Institute, Lucknow

The accessibility of *in vitro* cultured *P. falciparum* parasites has greatly increased their application in studies on basic biology and monitoring of chemotherapeutic responses. Although several species of avian, rodent and simian parasites have been used for *in vivo* studies, the *in vitro* models using nonhuman parasites have not been appropriately

exploited. We report successful long term *in vitro* cultivation of a simian parasite *Plasmodium knowlesi* in RPMI 1640 medium supplemented with 25mm HEPES 1% glucose, 0.23% $NaHCO_3$, and 15% normal monkey serum. The parasites were grown in 25mm diameter glass vials as well as in canted neck flasks and incubated at 37°C in a candle jar. The medium was changed every day and uninfected red cells were used to dilute the cultures every 2nd-4th day. An average of 2-5 fold increase was recorded every 72 hours. The parasites showed nearly 70,000 fold cumulative increase during a five week period and the viability of culture derived parasites was confirmed by inoculating a naive rhesus monkey. Conditions have been optimized for *in vitro* cultivation and proportional to the percentage of serum in the medium. Attempts to cryopreserve culture adapted parasites and reinstate long term cultures have been successful. In view of the quotidian periodicity, *Plasmodium knowlesi* parasites seem to be more suitable to lead identification of novel chemotherapeutic agents. In addition the cultures have been used to study the kinetics of antimalarial activity by determining ex vivo parasiticidal effect of drugs in serum samples obtained at periodical intervals after administration to rhesus monkey. Such an assay would provide an insight of pharmacodynamics of potential antimalarials to determine appropriate dosage schedules.

PS - 17.5

LEISHMANIA DONOVANI IN INDIAN LANGUR (*PRESBYTIS ENTELLUS*)I : AN ATTEMPT TO ESTABLISH DISEASE MODEL THROUGH NATURAL ROUTE

Alka Misra[1], P Sharma[1], J K Srivastava[1], Anuradha[1], J C Katiyar[1] and S Naik[2]
1. Division of Parasitology, CDRI, Lucknow, India.
2. Department of Immunology, SGPGI, Lucknow, India.

Indian langur monkeys (*Presbytis entellus*) were found to be highly susceptible to *L.donovani* infection when inoculated with amastigotes intravenously. To simulate natural route of infection, the langurs were infected intradermally with metacyclic promastigotes either alone or with one half lobe of sandfly salivary gland (s.g.) lysate. Sandfly saliva is reported to enhance infectivity of *Leishmania* promastigotes. The parasitic assessment done by splenic biopsy on day 60 post infection (p.i.) revealed rather low parasitic burden (1 to 4 amastigotes /1000 cell nuclei) in 50% monkeys infected with promastigotes along with s.g. lysate and this picture persisted till day 240 p.i. In group of monkeys infected with promastigote alone, no parasites could be demonstrated. In both the groups of monkeys no perceptible change in haematological and biochemical parameters or body temperature was noticed. However, there was a significant rise in antibody titre in monkeys of lysate group, which became apparent by 4 weeks p.i. and persisted till day 240 p.i. Both groups of langurs exhibited absence of proliferative response to specific *L. donovani* antigen from beginning up to day 150 p.i. However, by day 210 p.i. 2 monkeys from lysate group did show considerable increase in proliferative response.

Thus, single intradermal injection of promastigotes is not sufficient to establish infec-

tion. Multiple inoculations with enhanced dose of sandfly s.g. lysate are likely to yield positive results. These are presently under evaluation.

PS - 17.6

MONITORING OF DRUG SENSITIVITY LEVEL OF MULTI-DRUG RESISTANT STRAIN OF *PLASMODIUM YOELII* BY USING *IN VITRO* MICROTECHNIQUE.

Meenu Mishra, Renu Tripathi, Rashmi Agrawal and G.P. Dutta
Division of Microbiology, Central Drug Research Institute, Lucknow-226 001, INDIA.

Plasmodium yoelii nigeriensis is a multiple drug resistant malaria parasite of rodents which produces 100% mortality in swiss mice. The inhibitory effect of chloroquine was determined on MDR strain of *P.yoelii nigeriensis* by using *in vitro* microtechnique. Chloroquine was dissolved in DMSO and diluted with RPMI to stock concentration $1x10^{-3}$ mol/1. To determine the *in vitro* efficacy on culture of *P. yoelii,* serial dilutions were prepared at concentration ranging from $1x10^{-10}$ to $1x10^{-4}$ mol/l. Each dilution was tested in triplicate. Our results demonstrate that the value of IC_{50} for chloroquine is 0.1 µmol/l and chloroquine showed inhibitory effect on trophozoite and schizonts and achieving growth inhibition within 8-9hr. The used system is useful as primary screening test to select potential antimalarial compounds which could be subjected to *in vivo* screening in mouse model. Although it is a rodent parasite but it has limited advantages over *P.falciparum* screen since the result of *in vivo* and *in vitro* screening against the same MDR strain can be compared. Using this test system, the antimalarial activity of artemisinin and its derivatives have been evaluated and data will be discussed.

PS - 17.7

STUDIES ON CRYOPRESERVATION OF MALARIA PARASITES

C R Pillai, C Usha Devi and V P Sharma
Malaria Research Centre, Delhi

It is already known that the parasites can be cryopreserved in liquid nitrogen for a very long period. Since it is difficult to get liquid nitrogen in field conditions, alternative techniques are to be developed. The present study was undertaken to see the effect of different temperature conditions on the parasites preserved in different cryoprotectants viz. Sorbitol-glycerine and Me_2SO (Dimethyl sulfoxide, DMSO). The temperatures selected for comparison were room temperature (+25°C) refrigerator (+4°C) refrigerator Freezer chest (-10°C), deep freezer (-20°C and -70°C), and liquid nitrogen (-196°C). Pooled, adapted *P. falciparum* parasite aliquots with cryoprotectants kept at different temperatures were revived at different intervals to see their viability. The studies showed that the parasites could be kept viable in lower temperatures ranging from +4°C to -70°C for varying periods. The parasites preserved at +4°C and at -10°C could be revived up to 6th day of their preservation. Parasites kept at -70°C were revived even after 4

weeks without any problem. Parasites in sorbitol - glycerine kept at room temperature could not be revived, but parasites preserved in Me_2SO (DMSO) at room temperature could be revived even upto 4th day.

PS - 17.8

IN VITRO CULTIVATION OF TISSUE STAGES OF MALARIA PARASITE

Kamal K Kamboj
Division of Parasitology, Central Drug Research Institute, Lucknow-226001. India

The tissue stage of malaria parasite has often been described as "hidden" or the "silent" forms in the life cycle of the malaria parasite. These forms remained a mystery for many years and were discovered as late as 1948. Even after their discovery, they remained inaccesible to researchers because of their location in a deeper organ, the liver. These were also earlier thought to be free from host immune attack.

The discovery that human hepatoma cell line (HepG2) supports invasion of malaria sporozoites and their intracellular development greatly facilitated research on these "hidden" stages. Further, the development of methods for the isolation of viable hepatocytes by enzymatic perfusion and their successful maintenance in primary cultures have allowed studies on these forms int he homologous host cells.

The in vitro systems can be suitably employed to act as primary screen systems for evaluating the efficacy of potential tissue schizontocidals, candidate vaccines, cytokines etc. .

PS - 17.9

A GIARDIA LECTIN WITH BIOLOGIC ACTIVITY

H Sandhu, H Kaur, S Ghosh and N K Ganguly
Deptt of Experimental Medicine and Biotechnology, PGIMER, Chandigarh

The studies on the host parasite interactions involving the lectins have highlighted the importance of lectins as valuable tools to study the pathogenesis of the infection.

A panorama of membrane surface lectins have been reported in *G lamblia* which might play a vital role in recognition and adhesion of the parasite to intestinal epithelium and may directly contribute to subsequent microvillous damage.

A difference in the lectin activity of xenic trophozoites (obtained from small intestine of infected mouse) and axenic trophozoites (obtained from *in vitro* culture) suggests that xenic lectin might already be in an activated form as *G. lamblia* thrives in the proximal part of small intestine where trophozoites are exposed to host proteases and bacterial flora as well.

G. lamblia has a complex array of surface antigens and some of these are known to be excreted/secreted by the parasite and referred as Excretory/Secretory (ES) products.

The $(NH_4)_2SO_4$ precipitated E/S fraction of axenic *G. lamblia* grown *in vitro* was purified in the FPLC system. The ES product was a 58 KDa protein, thermolabile in nature and sensitive to pepsin, trypsin and pronase. It was characterized to be a glycoprotein in nature and lectin binding studies to Con A, PWM and PHA revealed x-methyl D-mannoside, n-acetyl- β-D-glucosamine and n-acetly B-D-galactosamine residues in carbohydrate portion of the protein.

The oral immunization of experimental animals led to complete failure of *G. lamblia* colonization on subsequent trophozoite inoculation. The immunization of mice also resulted in a significant stimulation of local as well as the systemic lymphoid system. The ESP has also revealed toxic activity as seen by rabbit ileal loop assay.

PS - 18.1

AN IMPROVED METHOD FOR THE STAINING OF LEISHMANIAL AMASTIGOTES

N L Pal, P Sharma, Anuradha and J C Katiyar
Division of Parasitology, Central Drug Research Institute, Lucknow - 226001, India.

Advance technology replaces the older and outdated ones. The conventional Giemsa method for the staining of intracellular parasites has been modified for special purpose by previous workers such as May-Grunwald Giemsa and Giemsa-Colophonium method for protozoan cysts in tissue sections.

The conventional Giemsa staining method fails to demonstrate the cytoplasmic components of leishmania amastigotes in the dab smears of spleen, bone-marrow etc. To view the cellular outline of the parasite clearly, an attempt was made to improve the staining technique by using a mordant solution. Before fixation, the air dried smears were washed with a mixture of aqueous solution of 1% potassium metabisulphite and 1.5% potassium dichromate for 50 seconds. This was followed by thorough washing in tap water. The smears were then semi-dried at room temperature and fixed with absolute methanol. Thereafter 5% Giemsa solution was poured over the smears and allowed to stand for 30 minutes.

With the introduction of this new mordant solution, the amastigotes in the stained slides looked brighter and more clear with distinct cell boundary and cytoplasm. The improved method is useful because it will clearly demonstrate any doubtful structure or artifact which are often encountered during observation.

PS - 18.2

PREVALENCE OF TAENIASIS IN ENDEMIC AND NON ENDEMIC AREAS ALONG WITH OTHER INTESTINAL NEMATODES IN GOA

S Estibeiro, M J W Pinto, S Vohra, D D Motghare and M P Verenkar

The present study was carried out to determine the prevalence of Taeniasis in 2 villages in Goa, one of which is endemic (Agassaim), and the other which is not (Mandur). An association of other helminthic parasites (ova & cysts) was also sought for. 500 healthy participant randomly selected but matched for age, sex and religion in both groups, constituted the material for the study. Stool samples collected from these individuals were subjected for microscopic examination before and after concentration.

Active taeniasis in the endemic area of Agassaim was 7.4% (37/500) before concentration technique. With an additional 23 cases being detected after concentration, the overall prevalence of taeniasis was 12%. In the non-endemic area, no cases of taeniasis were detected before or after concentration technique, thus confirming the higher preva-

lence rate in areas where pork industry is carried out and butcheries are present.

In Mandur area (non endemic for Taeniasis), percentage prevalence of other helminthic parasites was 60.4% (302/500). 188 stools yielded ova of helminths before concentration, while the additional 114 stools showed the parasitic ova after concentration. In contrast the prevalence of these parasites in taenia endemic area of Agassaim was low to the tune of 14% (73/500). Amongst the intestinal nematodes, Ascaris lumbricoides ova predominated in both the areas followed by Trichuris trichura. The low prevalence of intestinal nematodes in Agassaim probably relates to the indiscriminate use of Albendazole owing to the higher awareness of this disease in that locality.

Having proved the prevalence of Taeniasis, this study is being extended to determine the prevalence of cysticercosis in these areas. The study is also extended to determine the prevalence of taeniasis in semiurban area.

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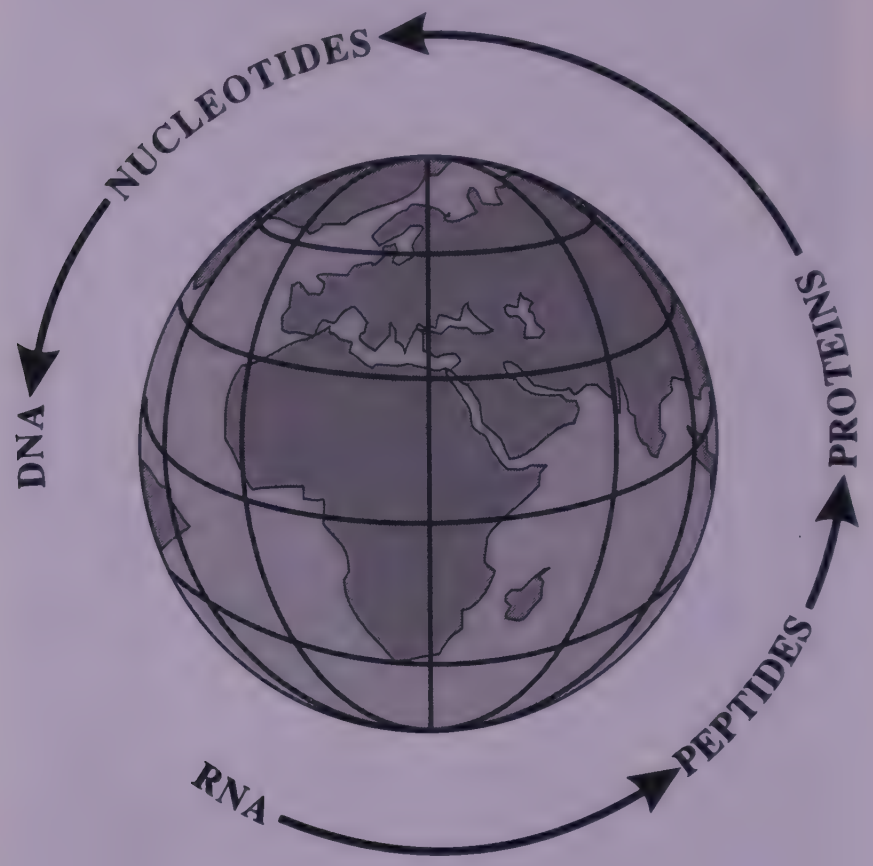

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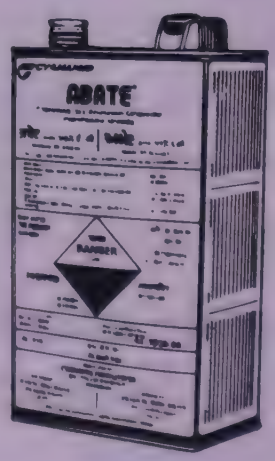

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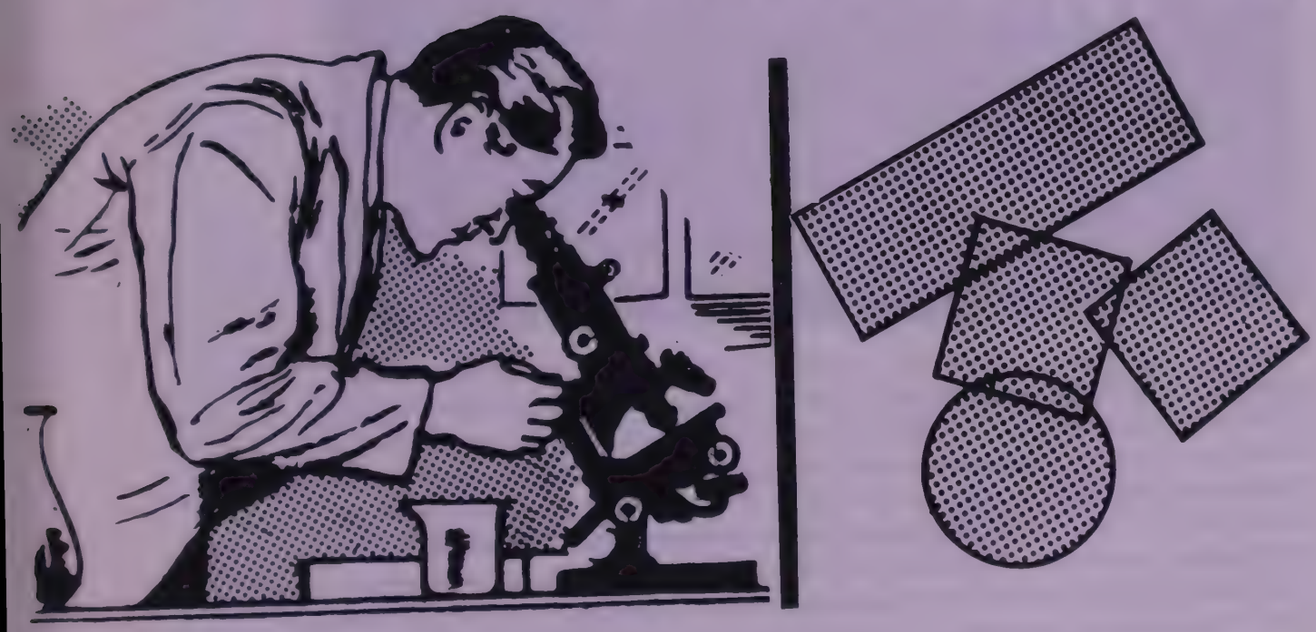

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(Pyrimethamine 25 mg + Sulphadoxine 500 mg)

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Abridged Prescribing Information : Indication : Malocide is indicated for the prophylaxis and treatment of malaria. Contraindications : Malocide is contraindicated in patients with hypersensitivity to pyrimethamine or sulfonamides. Patients with documented megaloblastic anemia due to folate deficiency. Infants less than two months of age. Prophylactic (repeated) use of Malocide is contraindicated in patients with severe renal insufficiency, marked liver parenchymal damage or blood dyscrasias. Adverse reactions : Malocide is usually well tolerated. Side effects in the form of gastrointestinal disturbances and skin rashes occur. Isolated cases of erythema multiforme (Stevens-Johnson syndrome) and toxic epidermal necrolysis have been reported. Blood dyscrasias may rarely occur over long periods of administration, hence regular blood counts are recommended during long-term prophylaxis. In this respect, particular care should be taken if treatment with other preparations containing folate inhibitors (e.g. cotrimoxazole, methotrexate, anticonvulsants) is given concomitantly. Drug Interactions : There have been reports indicating an increase in incidence and severity of adverse reactions when chloroquine is used with Malocide as compared to the use of Malocide alone. Antifolic drugs such as sulfonamides or trimethoprim-sulfamethoxazole combination should not be used when the patient is receiving Malocide for antimalarial prophylaxis. Malocide has not been reported to interfere with antidiabetic agents. Doage and administration : Curative treatment of malaria : The appropriate amount of the drug is given in one single dose. This dose should not be repeated for at least seven days. Adults : 2 to 3 tablets. Children : 10-14 years : 2 tablets. 7-9 years : $1^1/_2$ tablets. 4-6 years : 1 tablet. Under 4 year : $^1/_2$ tablet. In very severe case of P.falciparum, quinine may be added, preferably parenterally. An adequate supply of fluids and electrolytes should be maintained. Prophylactic management: The following dose of Malocide should be taken every seven days : Adults : 1 tablet., Children : $^1/_4$ to $^3/_4$ tablets depending on the age. Presentation : Available in strips of 2 tablets. Each uncoated tablet contains Sulfadoxine IP 500 mg and Pyrimethamine IP 25 mg.

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While every possible care has been taken in the
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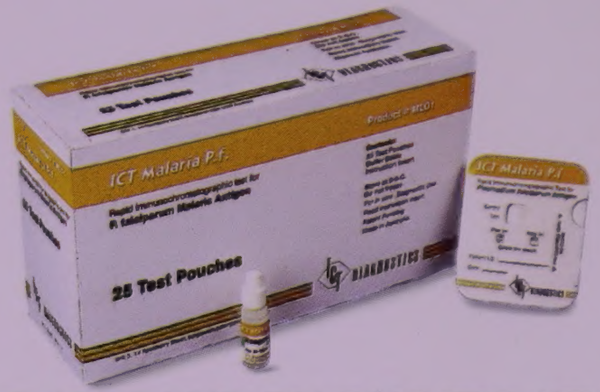

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ZENECA provides Double Protection

Malaria is a killer disease, transmitted by *Anopheles* mosquitoes, mainly in the tropics. Over 500 million people suffer from malaria annually, causing more than 2 million deaths every year. In many developing countries, malaria affects nearly everyone and the whole economy suffers. But we can prevent and control malaria.

Zeneca has developed anti-malaria medicines, for prevention and treatment of malaria cases, plus insecticides for mosquito control. Combinations of these products can be used effectively against drug-resistant strains of malaria and against insecticide-resistant strains of *Anopheles* mosquitoes.

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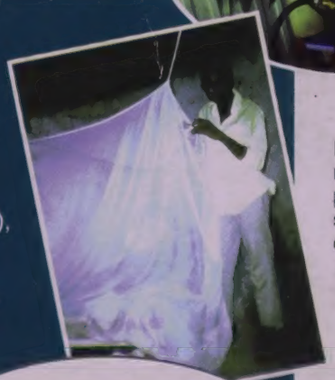

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